(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 May 2003 (01.05.2003)

PCT

(10) International Publication Number
WO 03/034812 A2

(51) International Patent Classification[7]: A01H

(21) International Application Number: PCT/US02/34079

(22) International Filing Date: 24 October 2002 (24.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/330,563 25 October 2001 (25.10.2001) US

(71) Applicant: MONSANTO TECHNOLOGY LLC [US/US]; 800 N. Lindbergh Boulevard, St. Louis, MO 63167 (US).

(72) Inventors: NORRIS, Susan, R.; 7701 Cornell Avenue, University City, MO 63130 (US). LINCOLN, Kim; 7249 Stanford Avenue, University City, MO 63130 (US). STEIN, Joshua, C.; 152 Nagog Hill Road, Acton, MA 01720 (US). VALENTIN, Henry, E.; 873 M Foxsprings Drive, Chesterfield, MO 63017 (US). VAN EENENNAAM, Alison; 856 Burr Street, Davis, CA 95616 (US).

(74) Agents: MARSH, David, R. et al.; ARNOLD & PORTER, Attn: IP Docketing Dept., Room 1126B, 555 Twelfth Street, N.W., Washington, DC 20004-1206 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/034812 A2

(54) Title: AROMATIC METHYLTRANSFERASES AND USES THEREOF

(57) Abstract: The present invention relates to genes associated with the tocopherol biosynthesis pathway. More particularly, the present invention provides and includes nucleic acid molecules, proteins, and antibodies associated with genes that encode polypeptides that have methyltransferase activity. The present invention also provides methods for utilizing such agents, for example in gene isolation, gene analysis and the production of transgenic plants. Moreover, the present invention includes transgenic plants modified to express the aforementioned polypeptides. In addition, the present invention includes methods for the production of products from the tocopherol biosynthesis pathway.

AROMATIC METHYLTRANSFERASES AND USES THEREOF

The present invention is in the field of plant genetics and biochemistry. More specifically, the invention relates to genes associated with the tocopherol biosynthesis pathway, namely those encoding methyltransferase activity, and uses of such genes.

Tocopherols are an important component of mammalian diets. Epidemiological evidence indicates that tocopherol supplementation can result in decreased risk for cardiovascular disease and cancer, can aid in immune function, and is associated with prevention or retardation of a number of degenerative disease processes in humans (Traber and Sies, *Annu. Rev. Nutr. 16*:321-347 (1996)). Tocopherol functions, in part, by stabilizing the lipid bilayer of biological membranes (Skrypin and Kagan, *Biochim. Biophys. Acta 815*:209 (1995); Kagan, *N.Y. Acad. Sci.* p 121, (1989); Gomez-Fernandez *et al., Ann. N.Y. Acad. Sci.* p 109 (1989)), reducing polyunsaturated fatty acid (PUFA) free radicals generated by lipid oxidation (Fukuzawa *et al., Lipids 17*:511-513 (1982)), and scavenging oxygen free radicals, lipid peroxy radicals and singlet oxygen species (Diplock *et al. Ann. N Y Acad. Sci.* 570:72 (1989); Fryer, *Plant Cell Environ. 15(4)*:381-392 (1992)).

The compound α-tocopherol, which is often referred to as vitamin E, belongs to a class of lipid-soluble antioxidants that includes α, β, γ, and δ-tocopherols and α, β, γ, and δ-tocotrienols. Although α, β, γ, and δ-tocopherols and α, β, γ, and δ-tocotrienols are sometimes referred to collectively as "vitamin E", vitamin E is more appropriately defined chemically as α-tocopherol. Vitamin E, or α-tocopherol, is significant for human health, in part because it is readily absorbed and retained by the body, and therefore has a higher degree of bioactivity than other tocopherol species (Traber and Sies, *Annu. Rev. Nutr. 16*:321-347 (1996)). However, other tocopherols such as β, γ, and δ-tocopherols also have significant health and nutritional benefits.

Tocopherols are primarily synthesized only by plants and certain other photosynthetic organisms, including cyanobacteria. As a result, mammalian dietary

tocopherols are obtained almost exclusively from these sources. Plant tissues vary considerably in total tocopherol content and tocopherol composition, with α-tocopherol the predominant tocopherol species found in green, photosynthetic plant tissues. Leaf tissue can contain from 10-50 μg of total tocopherols per gram fresh weight, but most of the world's major staple crops (*e.g.*, rice, corn, wheat, potato) produce low to extremely low levels of total tocopherols, of which only a small percentage is α-tocopherol (Hess, Vitamin E, α-tocopherol, *Antioxidants in Higher Plants*, R. Alscher and J. Hess, Eds., CRC Press, Boca Raton. pp. 111-134 (1993)). Oil seed crops generally contain much higher levels of total tocopherols, but α-tocopherol is present only as a minor component in most oilseeds (Taylor and Barnes, *Chemy Ind., Oct*:722-726 (1981)).

The recommended daily dietary intake of 15-30 mg of vitamin E is quite difficult to achieve from the average American diet. For example, it would take over 750 grams of spinach leaves, in which α-tocopherol comprises 60% of total tocopherols, or 200-400 grams of soybean oil to satisfy this recommended daily vitamin E intake. While it is possible to augment the diet with supplements, most of these supplements contain primarily synthetic vitamin E, having eight stereoisomers, whereas natural vitamin E is predominantly composed of only a single isomer. Furthermore, supplements tend to be relatively expensive, and the general population is disinclined to take vitamin supplements on a regular basis. Therefore, there is a need in the art for compositions and methods that either increase the total tocopherol production or increase the relative percentage of α-tocopherol produced by plants.

In addition to the health benefits of tocopherols, increased α-tocopherol levels in crops have been associated with enhanced stability and extended shelf life of plant products (Peterson, *Cereal-Chem. 72(1)*:21-24 (1995); Ball, *Fat-soluble vitamin assays in food analysis. A comprehensive review,* London, Elsevier Science Publishers Ltd. (1988)). Further, tocopherol supplementation of swine, beef, and poultry feeds has been shown to significantly increase meat quality and extend the shelf life of post-processed meat products by retarding post-processing lipid oxidation, which contributes to the undesirable flavor components (Sante and Lacourt, *J. Sci. Food Agric. 65(4)*:503-507 (1994); Buckley *et al., J. of Animal Science 73*:3122-3130 (1995)).

TOCOPHEROL BIOSYNTHESIS

The plastids of higher plants exhibit interconnected biochemical pathways leading to secondary metabolites including tocopherols. The tocopherol biosynthetic pathway in higher plants involves condensation of homogentisic acid and phytylpyrophosphate to form 2-methylphytylplastoquinol (Fiedler *et al.*, *Planta 155*:511-515 (1982); Soll *et al.*, *Arch. Biochem. Biophys. 204*:544-550 (1980); Marshall *et al.*, *Phytochem. 24*:1705-1711 (1985)). This plant tocopherol pathway can be divided into four parts: 1) synthesis of homogentisic acid (HGA), which contributes to the aromatic ring of tocopherol; 2) synthesis of phytylpyrophosphate, which contributes to the side chain of tocopherol; 3) joining of HGA and phytylpyrophosphate via a prenyltransferase followed by a subsequent cyclization; 4) and S-adenosyl methionine dependent methylation of an aromatic ring, which affects the relative abundance of each of the tocopherol species. See Figure 1.

Various genes and their encoded proteins that are involved in tocopherol biosynthesis are listed in the table below.

Gene ID or Enzyme Abbreviation	**Enzyme name**
tyrA	Bifunctional Prephenate dehydrogenase
HPT	Homogentisate phytyl transferase
DXS	1-Deoxyxylulose-5- phosphate synthase
DXR	1-Deoxyxylulose-5- phosphate reductoisomerase
GGPPS	Geranylgeranyl pyrophosphate synthase
HPPD	p-Hydroxyphenylpyruvate dioxygenase
AANT1	Adenylate transporter
IDI	Isopentenyl diphosphate isomerase
MT1	Methyl transferase 1
tMT2	Tocopherol methyl transferase 2
GGH	Geranylgeranyl diphosphate reductase
slr1737	Tocopherol cyclase
GMT	Gamma Methyl Transferase

As used herein, homogentisate phytyl transferase (HPT), phytylprenyl transferase (PPT), slr1736, and ATPT2, each refer to proteins or genes encoding proteins that have the same enzymatic activity.

SYNTHESIS OF HOMOGENTISIC ACID

Homogentisic acid is the common precursor to both tocopherols and plastoquinones. In at least some bacteria the synthesis of homogentisic acid is reported to occur via the conversion of chorismate to prephenate and then to p-hydroxyphenylpyruvate via a bifunctional prephenate dehydrogenase. Examples of bifunctional bacterial prephenate dehydrogenase enzymes include the proteins encoded by the *tyrA* genes of *Erwinia herbicola* and *Escherichia coli*. The *tyrA* gene product catalyzes the production of prephenate from chorismate, as well as the subsequent dehydrogenation of prephenate to form p-hydroxyphenylpyruvate (p-HPP), the immediate precursor to homogentisic acid. p-HPP is then converted to homogentisic acid by hydroxyphenylpyruvate dioxygenase (HPPD). In contrast, plants are believed to lack prephenate dehydrogenase activity, and it is generally believed that the synthesis of homogentisic acid from chorismate occurs via the synthesis and conversion of the intermediate arogenate. Since pathways involved in homogentisic acid synthesis are also responsible for tyrosine formation, any alterations in these pathways can also result in the alteration in tyrosine synthesis and the synthesis of other aromatic amino acids.

SYNTHESIS OF PHYTYLPYROPHOSPHATE

Tocopherols are a member of the class of compounds referred to as the isoprenoids. Other isoprenoids include carotenoids, gibberellins, terpenes, chlorophyll and abscisic acid. A central intermediate in the production of isoprenoids is isopentenyl diphosphate (IPP). Cytoplasmic and plastid-based pathways to generate IPP have been reported. The cytoplasmic based pathway involves the enzymes acetoacetyl CoA thiolase, HMGCoA synthase, HMGCoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

Recently, evidence for the existence of an alternative, plastid based, isoprenoid biosynthetic pathway emerged from studies in the research groups of Rohmer and Arigoni (Eisenreich *et al.*, *Chem. Bio.*, *5*:R221-R233 (1998); Rohmer, *Prog. Drug. Res.*, *50*:135-154 (1998); Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi (eds.), Pergamon Press, Oxford, England (1999)), who found that the isotope labeling patterns observed in studies on certain eubacterial and plant terpenoids could not be explained in terms of the mevalonate pathway. Arigoni and coworkers

subsequently showed that 1-deoxyxylulose, or a derivative thereof, serves as an intermediate of the novel pathway, now referred to as the MEP pathway (Rohmer *et al.*, *Biochem. J.*, *295*:517-524 (1993); Schwarz, Ph.D. thesis, Eidgenössiche Technische Hochschule, Zurich, Switzerland (1994)). Recent studies showed the formation of 1-deoxyxylulose 5-phosphate (Broers, Ph.D, thesis (Eidgenössiche Technische Hochschule, Zurich, Switzerland) (1994)) from one molecule each of glyceraldehyde 3-phosphate (Rohmer, *Comprehensive Natural Products Chemistry*, Vol. 2, pp. 45-68, Barton and Nakanishi, eds., Pergamon Press, Oxford, England (1999)) and pyruvate (Eisenreich *et al.*, *Chem. Biol.*, *5*:R223-R233 (1998); Schwarz *supra*; Rohmer *et al.*, *J. Am. Chem. Soc.*, *118*:2564-2566 (1996); and Sprenger *et al.*, *Proc. Natl. Acad. Sci. USA*, *94*:12857-12862 (1997)) by an enzyme encoded by the *dxs* gene (Lois *et al.*, *Proc. Natl. Acad. Sci. USA*, *95*:2105-2110 (1997); and Lange *et al.*, *Proc. Natl. Acad. Sci. USA*, *95*:2100-2104 (1998)). 1-Deoxyxylulose 5-phosphate can be further converted into 2-*C*-methylerythritol 4-phosphate (Arigoni *et al.*, *Proc. Natl. Acad. Sci. USA*, *94*:10600-10605 (1997)) by a reductoisomerase encoded by the *dxr* gene (Bouvier *et al.*, *Plant Physiol*, *117*:1421-1431 (1998); and Rohdich *et al.*, *Proc. Natl. Acad. Sci. USA*, *96*:11758-11763 (1999)).

Reported genes in the MEP pathway also include *ygbP*, which catalyzes the conversion of 2-C-methylerythritol 4-phosphate into its respective cytidyl pyrophosphate derivative and *ygbB*, which catalyzes the conversion of 4-phosphocytidyl-2C-methyl-D-erythritol into 2C-methyl-D-erythritol, 3, 4-cyclophosphate. These genes are tightly linked on the *E. coli* genome (Herz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *97(6)*:2485-2490 (2000)).

Once IPP is formed by the MEP pathway, it is converted to GGDP by GGDP synthase, and then to phytylpyrophosphate, which is the central constituent of the tocopherol side chain.

COMBINATION AND CYCLIZATION

Homogentisic acid is combined with either phytyl-pyrophosphate or solanyl-pyrophosphate by phytyl/prenyl transferase forming 2-methylphytyl plastoquinol or 2-methylsolanyl plastoquinol, respectively. 2-methylsolanyl plastoquinol is a precursor to the biosynthesis of plastoquinones, while 2-methylphytyl plastoquinol is ultimately converted to tocopherol.

METHYLATION OF THE AROMATIC RING

The major structural difference between each of the tocopherol subtypes is the position of the methyl groups around the phenyl ring. Both 2-methylphytyl plastoquinol and 2-methylsolanyl plastoquinol serve as substrates for the plant enzyme 2-methylphytylplatoquinol/2-methylsolanylplastoquinol methyltransferase (Tocopherol Methyl Transferase 2; Methyl Transferase 2; MT2; tMT2), which is capable of methylating a tocopherol precursor. Subsequent methylation at the 5 position of γ-tocopherol by γ-tocopherol methyl-transferase (GMT) generates the biologically active α-tocopherol.

A possible alternate pathway for the generation of α-tocopherol involves the generation of δ-tocopherol via the cyclization of 2-methylphytylplastoquinol by tocopherol cyclase. δ-tocopherol is then converted to β-tocopherol via the methylation of the 5 position by GMT. δ-tocopherol can be converted to α-tocopherol via methylation of the 3 position by tMT2, followed by methylation of the 5 position by GMT. In a possible alternative pathway, β-tocopherol is directly converted to α-tocopherol by tMT2 via the methylation of the 3 position (see, for example, *Biochemical Society Transactions, 11*:504-510 (1983); *Introduction to Plant Biochemistry*, 2nd edition, chapter 11 (1983); *Vitamin Hormone, 29*:153-200 (1971); *Biochemical Journal, 109*:577 (1968); and, *Biochemical and Biophysical Research Communication, 28(3)*:295 (1967)). Since all potential mechanisms for the generation of α-tocopherol involve catalysis by tMT2, plants that are deficient in this activity accumulate δ-tocopherol and β-tocopherol. Plants which have increased tMT2 activity tend to accumulate γ-tocopherol and α-tocopherol. Since there is no GMT activity in the seeds of many plants, these plants tend to accumulate γ-tocopherol.

There is a need in the art for nucleic acid molecules encoding enzymes involved in tocopherol biosynthesis, as well as related enzymes and antibodies for the enhancement or alteration of tocopherol production in plants. There is a further need for transgenic organisms expressing those nucleic acid molecules involved in tocopherol biosynthesis, which are capable of nutritionally enhancing food and feed sources.

BRIEF SUMMARY OF THE INVENTION

The present invention includes and provides a substantially purified nucleic acid molecule encoding a tMT2 enzyme.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 2.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3 through 7.

The present invention includes and provides a substantially purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 8 through 14.

The present invention includes and provides a substantially purified nucleic acid molecule encoding a plant polypeptide molecule having 2-Methylphytylplastoquinol methyltransferase activity.

The present invention includes and provides a substantially purified plant polypeptide molecule having 2-Methylphytylplastoquinol methyltransferase activity.

The present invention includes and provides a substantially purified mutant polypeptide molecule having an altered 2-Methylphytylplastoquinol methyltransferase activity relative to a non-mutant polypeptide.

The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 28.

The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21 and 29 through 32.

The present invention includes and provides a substantially purified polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 22 through 27 and 33 through 38.

The present invention includes and provides an antibody capable of specifically binding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38.

The present invention includes and provides a transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

The present invention includes and provides a transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

The present invention includes and provides a transformed plant comprising a nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and 29 through 32.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an

amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

The present invention includes and provides a method for reducing expression of the tMT2 gene in a plant comprising: (A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an introduced promoter region which functions in plant cells to cause the production of a mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

The present invention includes and provides a transformed plant comprising a nucleic acid molecule comprising an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence.

The present invention includes and provides a method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased α-tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

The present invention includes and provides a method of producing a plant having a seed with an increased α-tocopherol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule comprising a sequence selected from the group

consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 8 through 15.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced nucleic acid molecule comprising an introduced first nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a seed derived from a transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof, a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group

consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI, GGH*, and complements thereof.

The present invention includes and provides a transformed plant comprising an introduced first nucleic acid molecule encoding a tMT2 enzyme, and a second nucleic acid molecule encoding a GMT enzyme.

The present invention includes and provides a method of producing a plant having seed with an increased α-tocopherol level comprising: (A) transforming said plant with a nucleic acid molecule encoding a tMT2 enzyme and a nucleic acid molecule encoding a GMT enzyme; and (B) growing said plant.

BRIEF DESCRIPTION OF THE NUCLEIC AND AMINO ACID SEQUENCES

SEQ ID NO: 1 sets forth a nucleic acid sequence of a DNA molecule that encodes a wild type *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 2 sets forth a nucleic acid sequence of a DNA molecule that encodes a wild type *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 3 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt2 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 4 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt6 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 5 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt9 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 6 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt10 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 7 sets forth a nucleic acid sequence of a DNA molecule that encodes an hdt16 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 8 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Zea mays* tMT2 enzyme.

SEQ ID NO: 9 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 10 sets forth a nucleic acid sequence of a DNA molecule that encodes

an *Allium porrum* tMT2 enzyme.

SEQ ID NO: 11 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* tMT2 enzyme.

SEQ ID NO: 12 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Oryza sativa* tMT2 enzyme.

SEQ ID NO: 13 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* tMT2 enzyme.

SEQ ID NO: 14 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* tMT2 enzyme different in sequence from SEQ ID NO: 13.

SEQ ID NO: 15 sets forth a nucleic acid coding sequence of a wild type *Arabidopsis thaliana* tMT2 enzyme.

SEQ ID NO: 16 sets forth an amino acid sequence of a wild type *Arabidopsis thaliana*, Columbia and Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 17 sets forth an amino acid sequence of an hdt2 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 18 sets forth an amino acid sequence of an hdt6 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 19 sets forth an amino acid sequence of an hdt9 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 20 sets forth an amino acid sequence of an hdt10 mutant of the *Arabidopsis thaliana*, Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 21 sets forth an amino acid sequence of an hdt16 mutant of the *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 22 sets forth an amino acid sequence of a *Zea mays* tMT2 enzyme.

SEQ ID NO: 23 sets forth an amino acid sequence of a *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 24 sets forth an amino acid sequence of an *Allium porrum* tMT2 enzyme.

SEQ ID NO: 25 sets forth an amino acid sequence of a *Glycine max* tMT2 enzyme.

SEQ ID NO: 26 sets forth an amino acid sequence of an *Oryza sativa* tMT2 enzyme.

SEQ ID NO: 27 sets forth an amino acid sequence of a *Brassica napus* tMT2 enzyme.

SEQ ID NO: 28 sets forth an amino acid sequence of a mature wild type *Arabidopsis thaliana*, Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 29 sets forth an amino acid sequence of a mature hdt2 mutant of the *Arabidopsis thaliana,* Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 30 sets forth an amino acid sequence of a mature hdt6 mutant of the *Arabidopsis thaliana,* Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 31 sets forth an amino acid sequence of a mature hdt10 mutant of the *Arabidopsis thaliana,* Landsberg ecotype, tMT2 enzyme.

SEQ ID NO: 32 sets forth an amino acid sequence of a mature hdt16 mutant of the *Arabidopsis thaliana,* Columbia ecotype, tMT2 enzyme.

SEQ ID NO: 33 sets forth an amino acid sequence of a mature *Brassica napus* tMT2 enzyme.

SEQ ID NO: 34 sets forth an amino acid sequence of a mature *Oryza sativa* tMT2 enzyme.

SEQ ID NO: 35 sets forth an amino acid sequence of a mature *Zea mays* tMT2 enzyme.

SEQ ID NO: 36 sets forth an amino acid sequence of a mature *Glycine max* tMT2 enzyme.

SEQ ID NO: 37 sets forth an amino acid sequence of a mature *Allium porrum* tMT2 enzyme.

SEQ ID NO: 38 sets forth an amino acid sequence of a mature *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NO: 39 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana* γ-tocopherol methyltransferase.

SEQ ID NO: 40 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Arabidopsis thaliana,* Columbia ecotype, γ-tocopherol methyltransferase.

SEQ ID NO: 41 sets forth a nucleic acid sequence of a DNA molecule that encodes an *Oryza sativa* γ-tocopherol methyltransferase.

SEQ ID NO: 42 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Zea mays* γ-tocopherol methyltransferase.

SEQ ID NO: 43 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Gossypium hirsutum* γ-tocopherol methyltransferase.

SEQ ID NO: 44 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Cuphea pulcherrima* γ-tocopherol methyltransferase.

SEQ ID NO: 45 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* S8 γ-tocopherol methyltransferase.

SEQ ID NO: 46 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Brassica napus* P4 γ-tocopherol methyltransferase.

SEQ ID NO: 47 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Lycopersicon esculentum* γ-tocopherol methyltransferase.

SEQ ID NO: 48 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ-tocopherol methyltransferase 1.

SEQ ID NO: 49 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ-tocopherol methyltransferase 2.

SEQ ID NO: 50 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Glycine max* γ-tocopherol methyltransferase 3.

SEQ ID NO: 51 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Tagetes erecta* γ-tocopherol methyltransferase.

SEQ ID NO: 52 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Sorghum bicolor* γ-tocopherol methyltransferase

SEQ ID NO: 53 sets forth a nucleic acid sequence of a DNA molecule that encodes a *Nostoc punctiforme* γ-tocopherol methyltransferase.

SEQ ID NO: 54 sets forth a nucleic acid sequence of a DNA molecule that encodes

an *Anabaena* γ-tocopherol methyltransferase.

SEQ ID NOs: 55 and 56 set forth nucleic acid sequences of the MAA21_40_1 primer pair.

SEQ ID NOs: 57 and 58 set forth nucleic acid sequences of the MAA21_40_2 primer pair.

SEQ ID NOs: 59 and 60 set forth nucleic acid sequences of the MAA21_40_3 primer pair.

SEQ ID NOs: 61 and 62 set forth nucleic acid sequences of the MAA21_40_4 primer pair.

SEQ ID NOs: 63 and 64 set forth nucleic acid sequences of the MAA21_40_5 primer pair.

SEQ ID NOs: 65 and 66 set forth nucleic acid sequences of the MAA21_40_6 primer pair.

SEQ ID NOs: 67 and 68 set forth nucleic acid sequences of the MAA21_40_7 primer pair.

SEQ ID NOs: 69 and 70 set forth nucleic acid sequences of the MAA21_40_8 primer pair.

SEQ ID NOs: 71 and 72 set forth nucleic acid sequences of the MAA21_40_9 primer pair.

SEQ ID NOs: 73 and 74 set forth nucleic acid sequences of the MAA21_40_10 primer pair.

SEQ ID NOs: 75 and 76 set forth nucleic acid sequences of the MAA21_40_11 primer pair.

SEQ ID NOs: 77 and 78 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Brassica napus* tMT2 enzyme.

SEQ ID NOs: 79 and 80 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Oryza sativa* tMT2 enzyme.

SEQ ID NOs: 81 and 82 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Zea mays* tMT2 enzyme.

SEQ ID NOs: 83 and 84 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Glycine max* tMT2 enzyme.

SEQ ID NOs: 85 and 86 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Allium porrum* tMT2 enzyme.

SEQ ID NOs: 87 and 88 set forth nucleic acid sequences of primers for use in amplifying a gene encoding a mature *Gossypium hirsutum* tMT2 enzyme.

SEQ ID NOs: 89 and 90 set forth nucleic acid sequences of primers #17286 and #17181 for use in amplifying a gene encoding a full length *Arabidopsis* thaliana tMT2 enzyme.

SEQ ID NO: 91 sets forth an amino acid sequence of an *Arabidopsis thaliana* γ-tocopherol methyltransferase.

SEQ ID NO: 92 sets forth an amino acid sequence of an *Arabidopsis thaliana,* Columbia ecotype, γ-tocopherol methyltransferase.

SEQ ID NO: 93 sets forth an amino acid sequence of an *Oryza sativa* γ-tocopherol methyltransferase.

SEQ ID NO: 94 sets forth an amino acid sequence of a *Zea mays* γ-tocopherol methyltransferase.

SEQ ID NO: 95 sets forth an amino acid sequence of a *Gossypium hirsutum* γ-tocopherol methyltransferase.

SEQ ID NO: 96 sets forth an amino acid sequence of a *Cuphea pulcherrima* γ-tocopherol methyltransferase.

SEQ ID NO: 97 sets forth an amino acid sequence of a *Brassica napus* S8 γ-tocopherol methyltransferase.

SEQ ID NO: 98 sets forth an amino acid sequence of a *Brassica napus* P4 γ-tocopherol methyltransferase.

SEQ ID NO: 99 sets forth an amino acid sequence of a *Lycopersicon esculentum* γ-tocopherol methyltransferase.

SEQ ID NO: 100 sets forth an amino acid sequence of a *Glycine max* γ-tocopherol methyltransferase 1.

SEQ ID NO: 101 sets forth an amino acid sequence of a *Glycine max* γ-tocopherol methyltransferase 2.

SEQ ID NO: 102 sets forth an amino acid sequence of a *Glycine max* γ-tocopherol methyltransferase 3.

SEQ ID NO: 103 sets forth an amino acid sequence of a *Tagetes erecta* γ-tocopherol methyltransferase.

SEQ ID NO: 104 sets forth an amino acid sequence of a *Sorghum bicolor* γ-tocopherol methyltransferase.

SEQ ID NO: 105 sets forth an amino acid sequence of a *Lilium asiaticum* γ-tocopherol methyltransferase.

SEQ ID NO: 106 sets forth an amino acid sequence of a *Nostoc punctiforme* γ-tocopherol methyltransferase.

SEQ ID NO: 107 sets forth an amino acid sequence of an *Anabaena* γ-tocopherol methyltransferase.

tocopherol methyltransferase.

SEQ ID NO: 108 sets forth an amino acid consensus sequence for the aligned polypeptides shown in Figures 3a and 3b.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of the tocopherol biosynthetic pathway.

Figure 2 represents the results of a TBLASTN homology comparison of the nucleotide sequences of several crop tMT2 genes to the amino acid sequence of a tMT2 gene from *Arabidopsis thaliana* (NCBI General Identifier Number gi7573324).

Figures 3a and 3b represent the Pretty Alignment (Genetics Computer Group, Madison WI) of tMT2 protein sequences from different plant species.

Figure 4 represents a graph depicting the methyltransferase activity of recombinantly expressed *Anabaena* MT1 (positive control). Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

Figure 5 represents a graph depicting the methyltransferase activity of recombinantly expressed mature *Arabidopsis* tMT2. Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67191.

Figure 6 represents a graph depicting the methyltransferase activity of recombinantly expressed mature *Arabidopsis* tMT2 hdt2 mutant. Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67207.

Figure 7 represents a graph depicting the methyltransferase activity of recombinantly expressed *Anabaena* MT1 without 2-methylphytylplastoquinol substrate (negative control). Enzyme activity is monitored on crude cell extracts from *E. coli* harboring pMON67174.

Figure 8 represents a graph depicting the methyltransferase I activity in isolated pea chloroplasts (positive control).

Figure 9 is a plasmid map of pMON67205.

Figure 10 is a plasmid map of pMON67220.

Figure 11 is a plasmid map of pMON67226.

Figure 12 is a plasmid map of pMON67225.

Figure 13 is a plasmid map of pMON67227.

Figure 14 is a plasmid map of pMON67224.

Figure 15 is a plasmid map of pMON67223.

Figures 16a and 16b depict the levels of expression of δ-tocopherol in various types of *Arabidopsis*.

Figure 17 depicts T3 seed δ-tocopherol (%) from two lines expressing tMT2 under the control of the napin promoter (pMON67205) in the *hdt2* mutant line.

Figures 18a-d depict the levels of α, β, γ, and δ-tocopherol in tMT2 pools of 10 seeds.

Figures 19a-d depict the levels of α, β, γ, and δ-tocopherol in tMT2/GMT pools of 10 seeds.

Figure 20 depicts the tocopherol composition of single seeds from one line of soybean (28072) transformed with pMON67226.

Figures 21a-d depict the levels of α, β, γ, and δ-tocopherol in R1 Soy Single Seed from pMON67226.

Figure 22 depicts the tocopherol composition of single seeds from one line of soybean (28906) transformed with pMON67227.

Figures 23a-d depict the levels of α, β, γ, and δ-tocopherol in R1 Soy Single Seed from pMON67227.

Figure 24 depicts the results of various 2-methylphytylplastoquinol methyltransferase assays.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a number of agents, for example, nucleic acid molecules and polypeptides associated with the synthesis of tocopherol, and provides uses of such agents.

AGENTS

The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response. The agents will preferably be "substantially purified." The term "substantially purified," as used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native environmental conditions. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native environmental conditions.

The agents of the invention may also be recombinant. As used herein, the term recombinant means any agent (*e.g.*, DNA, peptide *etc.*), that is, or results, however indirectly, from human manipulation of a nucleic acid molecule.

It is understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent (*e.g.*, fluorescent labels, Prober *et al.*, *Science 238*:336-340

(1987); Albarella *et al.*, EP 144914; chemical labels, Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417; modified bases, Miyoshi *et al.*, EP 119448).

NUCLEIC ACID MOLECULES

Agents of the invention include nucleic acid molecules. In a preferred aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence, which encodes a tocopherol methyltransferase. As used herein, a tocopherol methyltransferase (tMT2) is any plant protein that is capable of specifically catalyzing the methylation of the 3 position of the phenyl ring of 2-methylphytylplastoquinol, 2-methyl-5-phytylplastoquinol, 2-methyl-3-phytylplastoquinol, δ-tocopherol, or β-tocopherol (see, *Photosyn. Research*, *31*:99-111 (1992) and *Phytochemistry 19*:215-218 (1980)). A preferred tMT2 is found in an organism selected from the group consisting of *Arabidopsis*, maize, cotton, leek, soybean, rice, and oilseed rape. An example of a more preferred tMT2 is a polypeptide with the amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38. In a more preferred embodiment, the tMT2 is encoded by any of SEQ ID NOs: 1 through 15.

In another preferred aspect of the present invention a nucleic acid molecule of the present invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In another preferred aspect of the present invention, a nucleic acid molecule of the present invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 and 2, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 3 through 7, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 8 through 14, and complements thereof. In another preferred aspect of the present invention the nucleic acid molecule of the invention comprises the nucleic acid sequence of SEQ ID NO: 15 and its complement. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding amino acid sequence SEQ ID NO: 16 and fragments thereof.

In another embodiment, the present invention provides nucleic acid molecules comprising a sequence encoding SEQ ID NO: 108, and complements thereof. In another aspect, the present invention provides nucleic acid molecules comprising a sequence encoding residues 83 through 356 of SEQ ID NO: 108, and its complement. In another aspect, the present invention provides nucleic acid molecules comprising a sequence encoding a fragment of residues 83 through 356 of SEQ ID NO: 108, wherein the fragment has a length of at least about 25, 50, 75, 100, 150, 200, or 250 residues, and complements thereof. In yet another aspect, the present invention provides nucleic acid molecules encoding one or more of the following fragments of SEQ ID NO: 108, and complements thereof: 82 through 123, 132 through 146, and 269 through 295.

The present invention includes the use of the above-described sequences and fragments thereof in transgenic plants, other organisms, and for other uses as described below.

In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid of SEQ ID NO: 28 and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof.

In another preferred aspect of the present invention a nucleic acid molecule comprises nucleotide sequences encoding a plastid transit peptide operably fused to a nucleic acid molecule that encodes a protein or fragment of the present invention.

In another preferred embodiment of the present invention, the nucleic acid molecules of the invention encode mutant tMT2 enzymes. As used herein, a "mutant" enzyme or polypeptide is any enzyme or polypeptide that contains an amino acid that is different from the amino acid in the same position of a wild type enzyme of the same type. Examples of suitable mutants of the invention include, but are not limited to, those found in Example 1 of this application.

It is understood that in a further aspect of nucleic acid sequences of the present invention, the nucleic acids can encode a protein that differs from any of the proteins in that one or more amino acids have been deleted, substituted or added without altering the function. For example, it is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

In one aspect of the present invention the nucleic acids of the present invention are said to be introduced nucleic acid molecules. A nucleic acid molecule is said to be "introduced" if it is inserted into a cell or organism as a result of human manipulation, no matter how indirect. Examples of introduced nucleic acid molecules include, without limitation, nucleic acids that have been introduced into cells via transformation, transfection, injection, and projection, and those that have been introduced into an organism via conjugation, endocytosis, phagocytosis, etc.

One subset of the nucleic acid molecules of the invention is fragment nucleic acids molecules. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, the nucleic acid molecules of the invention, such as those specifically disclosed. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 400 nucleotide residues and more preferably, about 15 to about 30 nucleotide residues, or about 50 to about 100 nucleotide residues, or about 100 to about 200 nucleotide residues, or about 200 to about 400 nucleotide residues, or about 275 to about 350 nucleotide residues).

A fragment of one or more of the nucleic acid molecules of the invention may be a probe and specifically a PCR probe. A PCR probe is a nucleic acid molecule capable of initiating a polymerase activity while in a double-stranded structure with another nucleic acid. Various methods for determining the structure of PCR probes and PCR techniques exist in the art. Computer generated searches using programs such as Primer3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), STSPipeline (www-

genome.wi.mit.edu/cgi-bin/www-STS_Pipeline), or GeneUp (Pesole *et al.*, *BioTechniques* *25*:112-123 (1998)), for example, can be used to identify potential PCR primers.

Nucleic acid molecules or fragments thereof of the present invention are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. Nucleic acid molecules of the present invention include those that specifically hybridize to nucleic acid molecules having a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof. Nucleic acid molecules of the present invention also include those that specifically hybridize to nucleic acid molecules encoding an amino acid sequence selected from SEQ ID NOs: 16 through 38, and fragments thereof.

As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989), and by Haymes *et al.*, *Nucleic Acid Hybridization, A Practical Approach, IRL Press*, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of

2.0 X SSC at 20-25°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 50°C to a high stringency of about 0.2 X SSC at 65°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1 through 15, and complements thereof under moderately stringent conditions, for example at about 2.0 X SSC and about 65°C.

In a particularly preferred embodiment, a nucleic acid of the present invention will include those nucleic acid molecules that specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1 through 15, and complements thereof under high stringency conditions such as 0.2 X SSC and about 65°C.

In one aspect of the present invention, the nucleic acid molecules of the present invention have one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, and complements thereof. In another aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 90% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 95% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, complements thereof, and fragments of either. In a more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 98% sequence identity with one or more of the nucleic acid sequences set forth in SEQ ID NOs: 1 through 15, complements thereof and fragments of either. In an even more preferred aspect of the present invention, one or more of the nucleic acid molecules of the present invention share between 100% and 99% sequence identity with one or more of the sequences set forth in SEQ ID NOs: 1 through 15, complements thereof, and fragments of either.

In a preferred embodiment the percent identity calculations are performed using BLASTN or BLASTP (default, parameters, version 2.0.8, Altschul *et al., Nucleic Acids Res. 25*:3389-3402 (1997)).

A nucleic acid molecule of the invention can also encode a homolog polypeptide. As used herein, a homolog polypeptide molecule or fragment thereof is a counterpart protein molecule or fragment thereof in a second species (*e.g.*, corn rubisco small subunit is a homolog of *Arabidopsis* rubisco small subunit). A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original polypeptide (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, *Brassica campestris, Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm. More particularly, preferred homologs are selected from canola, corn, *Brassica campestris, Brassica napus*, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, rapeseed, safflower, oil palm, flax, and sunflower. In an even more preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica napus*, oilseed rape, soybean, sunflower, safflower, oil palms, and peanut. In a particularly preferred embodiment, the homolog is soybean. In a particularly preferred embodiment, the homolog is canola. In a particularly preferred embodiment, the homolog is oilseed rape.

In a preferred embodiment, nucleic acid molecules having SEQ ID NOs: 1 through 15, complements thereof, and fragments of either; or more preferably SEQ ID NOs: 1 through 15, and complements thereof, can be utilized to obtain such homologs.

In another further aspect of the present invention, nucleic acid molecules of the present invention can comprise sequences that differ from those encoding a polypeptide or fragment thereof in SEQ ID NOs: 1 through 15 due to the fact that a polypeptide can have one or more conservative amino acid changes, and nucleic acid sequences coding for the

polypeptide can therefore have sequence differences. It is understood that codons capable of coding for such conservative amino acid substitutions are known in the art.

It is well known in the art that one or more amino acids in a native sequence can be substituted with other amino acid(s), the charge and polarity of which are similar to that of the native amino acid, *i.e.*, a conservative amino acid substitution. Conservative substitutes for an amino acid within the native polypeptide sequence can be selected from other members of the class to which the amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids, (2) basic amino acids, (3) neutral polar amino acids, and (4) neutral, nonpolar amino acids. Representative amino acids within these various groups include, but are not limited to, (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

Conservative amino acid substitution within the native polypeptide sequence can be made by replacing one amino acid from within one of these groups with another amino acid from within the same group. In a preferred aspect, biologically functional equivalents of the proteins or fragments thereof of the present invention can have ten or fewer conservative amino acid changes, more preferably seven or fewer conservative amino acid changes, and most preferably five or fewer conservative amino acid changes. The encoding nucleotide sequence will thus have corresponding base substitutions, permitting it to encode biologically functional equivalent forms of the polypeptides of the present invention.

It is understood that certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Because it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence and, of course, its underlying DNA coding sequence and, nevertheless, a protein with like properties can still be obtained. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the proteins or fragments of the present invention, or corresponding DNA sequences that encode said

peptides, without appreciable loss of their biological utility or activity. It is understood that codons capable of coding for such amino acid changes are known in the art.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol.* *157*, 105-132 (1982)). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *J. Mol. Biol. 157:*105-132 (1982)); these are isoleucine (+4.5), valine (+4.2), leucine (+3.8), phenylalanine (+2.8), cysteine/cystine (+2.5), methionine (+1.9), alanine (+1.8), glycine (-0.4), threonine (-0.7), serine (-0.8), tryptophan (-0.9), tyrosine (-1.3), proline (-1.6), histidine (-3.2), glutamate (-3.5), glutamine (-3.5), aspartate (-3.5), asparagine (-3.5), lysine (-3.9), and arginine (-4.5).

In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0), lysine (+3.0), aspartate (+3.0±1), glutamate (+3.0±1), serine (+0.3), asparagine (+0.2), glutamine (+0.2), glycine (0), threonine (-0.4), proline (-0.5±1), alanine (-0.5), histidine (-0.5), cysteine (-1.0), methionine (-1.3), valine (-1.5), leucine (-1.8), isoleucine (-1.8), tyrosine (-2.3), phenylalanine (-2.5), and tryptophan (-3.4).

In making such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those for which a specific sequence is provided herein because one or more codons has been replaced with a codon that encodes a conservative substitution of the amino acid originally encoded.

Agents of the invention include nucleic acid molecules that encode at least about a contiguous 10 amino acid region of a polypeptide of the present invention, more preferably at least about a contiguous 25, 40, 50, 100, or 125 amino acid region of a polypeptide of the present invention.

In a preferred embodiment, any of the nucleic acid molecules of the present invention can be operably linked to a promoter region that functions in a plant cell to cause the production of an mRNA molecule, where the nucleic acid molecule that is linked to the promoter is heterologous with respect to that promoter. As used herein, "heterologous" means not naturally occurring together.

PROTEIN AND PEPTIDE MOLECULES

A class of agents includes one or more of the polypeptide molecules encoded by a nucleic acid agent of the invention. A particular preferred class of proteins is that having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence encoding an amino acid of SEQ ID NO: 28 and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the polypeptide molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof.

In another embodiment, the present invention provides a polypeptide comprising the amino acid sequence of SEQ ID NO: 108. In another aspect, the present invention provides a polypeptide comprising the amino acid sequence of residues 83 through 356 of SEQ ID NO: 108. In another aspect, the present invention provides a polypeptide fragment comprising the amino acid sequence of residues 83 through 356 of SEQ ID NO: 108, wherein the fragment has a length of at least about 25, 50, 75, 100, 150, 200, or 250 residues. In yet another aspect, the present invention provides a polypeptide comprising the amino acid sequence of one or more of the following fragments of SEQ ID NO: 108: 82 through 123, 132 through 146, and 269 through 295.

Polypeptide agents may have C-terminal or N-terminal amino acid sequence extensions. One class of N-terminal extensions employed in a preferred embodiment are plastid transit peptides. When employed, plastid transit peptides can be operatively linked to the N-terminal sequence, thereby permitting the localization of the agent polypeptides to plastids. In an embodiment of the present invention, any suitable plastid targeting sequence can be used. Where suitable, a plastid targeting sequence can be substituted for a native plastid targeting sequence, for example, for the CTP occurring natively in the tMT2 protein. In a further embodiment, a plastid targeting sequence that is heterologous to any tMT2 protein or fragment described herein can be used. In a further embodiment, any suitable, modified plastid targeting sequence can be used. In another embodiment, the plastid targeting sequence is a CTP1 sequence (*see* WO 00/61771).

In a preferred aspect a protein of the present invention is targeted to a plastid using either a native transit peptide sequence or a heterologous transit peptide sequence. In the case of nucleic acid sequences corresponding to nucleic acid sequences of non-higher plant organisms such as cynobacteria, such nucleic acid sequences can be modified to attach the coding sequence of the protein to a nucleic acid sequence of a plastid targeting peptide.

As used herein, the term "protein," "peptide molecule," or "polypeptide" includes any molecule that comprises five or more amino acids. It is well known in the art that protein, peptide or polypeptide molecules may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation, glycosylation, phosphorylation, or oligomerization. Thus, as used herein, the term "protein," "peptide molecule," or "polypeptide" includes any protein that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer

to all naturally occurring L-amino acids. This definition is meant to include norleucine, norvaline, ornithine, homocysteine, and homoserine.

One or more of the protein or fragments thereof, peptide molecules, or polypeptide molecules may be produced via chemical synthesis, or more preferably, by expression in a suitable bacterial or eukaryotic host. Suitable methods for expression are described by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press*, Cold Spring Harbor, New York (1989) or similar texts.

A "protein fragment" is a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein. A protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein is a "fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin). Fusion protein or peptide molecules of the invention are preferably produced via recombinant means.

Another class of agents comprise protein, peptide molecules, or polypeptide molecules or fragments or fusions thereof comprising SEQ ID NOs: 16 through 38, and fragments thereof in which conservative, non-essential or non-relevant amino acid residues have been added, replaced or deleted. Computerized means for designing modifications in protein structure are known in the art (Dahiyat and Mayo, *Science* 278:82-87 (1997)).

A protein, peptide or polypeptide of the invention can also be a homolog protein, peptide or polypeptide. As used herein, a homolog protein, peptide or polypeptide or fragment thereof is a counterpart protein, peptide or polypeptide or fragment thereof in a second species. A homolog can also be generated by molecular evolution or DNA shuffling techniques, so that the molecule retains at least one functional or structure characteristic of the original (*see*, for example, U.S. Patent 5,811,238).

In another embodiment, the homolog is selected from the group consisting of alfalfa, *Arabidopsis*, barley, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, and *Phaseolus*. More particularly, preferred homologs are selected from canola, rapeseed, corn, *Brassica campestris, Brassica napus*, oilseed rape, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower. In an even more

preferred embodiment, the homolog is selected from the group consisting of canola, rapeseed, corn, *Brassica campestris, Brassica napus,* oilseed rape, soybean, sunflower, safflower, oil palms, and peanut. In a preferred embodiment, the homolog is soybean. In a preferred embodiment, the homolog is canola. In a preferred embodiment, the homolog is oilseed rape.

In a preferred embodiment, the nucleic acid molecules of the present invention or complements and fragments of either can be utilized to obtain such homologs.

Agents of the invention include proteins and fragments thereof comprising at least about a contiguous 10 amino acid region preferably comprising at least about a contiguous 20 amino acid region, even more preferably comprising at least about a contiguous 25, 35, 50, 75 or 100 amino acid region of a protein of the present invention. In another preferred embodiment, the proteins of the present invention include between about 10 and about 25 contiguous amino acid region, more preferably between about 20 and about 50 contiguous amino acid region, and even more preferably between about 40 and about 80 contiguous amino acid region.

PLANT CONSTRUCTS AND PLANT TRANSFORMANTS

One or more of the nucleic acid molecules of the invention may be used in plant transformation or transfection. Exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant. Exogenous genetic material is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism.

In a preferred aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence that encodes tocopherol methyltransferase. In another preferred aspect of the present invention the exogenous genetic material of the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and complements thereof and fragments of either. In a further aspect of the present invention the exogenous genetic material comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 through 21, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid

sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 22 through 27, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 through 38, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid of SEQ ID NO: 28, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 29 through 32, and fragments thereof. In a further aspect of the present invention the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOs: 33 through 38, and fragments thereof. In a further aspect of the present invention, the nucleic acid sequences of the invention also encode peptides involved in intracellular localization, export, or post-translational modification.

In an embodiment of the present invention, exogenous genetic material comprising a tMT2 enzyme or fragment thereof is introduced into a plant with one or more additional genes. In one embodiment, preferred combinations of genes include one or more of the following genes: *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, GMT, tMT2, AANT1, slr1737, IDI, GGH*, or a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991); Keegstra, *Cell 56(2)*:247-53 (1989); Nawrath, *et al., Proc. Natl. Acad. Sci. U.S.A. 91*:12760-12764 (1994); Xia *et al., J. Gen. Microbiol. 138*:1309-1316 (1992); Cyanobase, www.kazusa.or.jp/cyanobase; Lois *et al., Proc. Natl. Acad. Sci. U.S.A. 95 (5)*:2105-2110 (1998); Takahashi *et al. Proc. Natl. Acad. Sci. U.S.A. 95 (17)*, 9879-9884 (1998); Norris *et al., Plant Physiol. 117*:1317-1323 (1998); Bartley and Scolnik, *Plant Physiol. 104*:1469-1470 (1994), Smith *et al., Plant J. 11:8*3-92 (1997); WO 00/32757; WO 00/10380; Saint Guily, *et al., Plant Physiol., 100(2)*:1069-1071 (1992); Sato *et al., J. DNA Res. 7 (1)*:31-63 (2000)).

In another preferred embodiment, tMT2 is combined with *GMT*. In any of the embodiments disclosed herein in which a nucleic acid molecule encoding a *GMT* is used, the nucleic acid molecule is preferably selected from the group consisting of nucleic acid molecules comprising a nucleic acid sequence selected from the group SEQ ID NOs: 39 and 54, and nucleic acids molecules encoding GMTs having an amino acid sequence

selected from the group consisting of SEQ ID NOs: 39-54. In another preferred embodiment, tMT2 is combined with *GMT* and one or more of the genes listed above. In such combinations, one or more of the gene products can be directed to the plastid by the use of a plastid targeting sequence. Alternatively, one or more of the gene products can be localized in the cytoplasm. In a preferred aspect the gene products of *tyr*A and HPPD are targeted to the cytoplasm. Such genes can be introduced, for example, with the tMT2 or *GMT* or both, or fragment of either or both on a single construct, introduced on different constructs but the same transformation event, or introduced into separate plants followed by one or more crosses to generate the desired combination of genes. In such combinations, a preferred promoter is a napin promoter and a preferred plastid targeting sequence is a CTP1 sequence. It is preferred that gene products are targeted to the plastid.

In a preferred combination a nucleic acid molecule encoding a tMT2 polypeptide and a nucleic acid molecule encoding any of the following enzymes: *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, tMT2, AANT1, slr1737, IDI, GGH* or a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase are introduced into a plant. A particularly preferred combination that can be introduced is a nucleic acid molecule encoding a tMT2 polypeptide and a nucleic acid molecule encoding a *GMT* polypeptide, where both polypeptides are targeted to the plastid and where one of such polypeptides is present and the other is introduced. Both nucleic acid sequences encoding such polypeptides can be introduced using a single gene construct, or each polypeptide can be introduced on separate constructs. In a further embodiment, tMT2 is combined with *GMT* and one or more of *tyrA, slr1736, HPT tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI,* and *GGH*.

In a particularly preferred combination, a nucleic acid molecule encoding a tMT2 protein and a nucleic acid molecule encoding a GMT enzyme are introduced into a plant along with a nucleic acid molecule that encodes one or more of *tyrA, slr1736, HPT tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr1737, IDI,* and *GGH*.

Another particularly preferred combination that can be introduced is a nucleic acid molecule encoding a tMT2 protein and a nucleic acid molecule that results in the down regulation of a GMT protein. In such an aspect, it is preferred that the plant accumulates either γ-tocopherol or γ-tocotrienol or both.

Such genetic material may be transferred into either monocotyledons or dicotyledons including, but not limited to canola, corn, soybean, *Arabidopsis phaseolus*, peanut, alfalfa, wheat, rice, oat, sorghum, rapeseed, rye, tritordeum, millet, fescue, perennial ryegrass, sugarcane, cranberry, papaya, banana, safflower, oil palms, flax, muskmelon, apple, cucumber, dendrobium, gladiolus, chrysanthemum, liliacea, cotton, eucalyptus, sunflower, *Brassica campestris*, oilseed rape, turfgrass, sugarbeet, coffee and dioscorea (Christou, In: *Particle Bombardment for Genetic Engineering of Plants*, Biotechnology Intelligence Unit. Academic Press, San Diego, California (1996)), with canola, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax, and sunflower preferred, and canola, rapeseed, corn, *Brassica campestris*, *Brassica napus*, oilseed rape, soybean, sunflower, safflower, oil palms, and peanut preferred. In a more preferred embodiment, the genetic material is transferred into canola. In another more preferred embodiment, the genetic material is transferred into oilseed rape. In another particularly preferred embodiment, the genetic material is transferred into soybean.

Transfer of a nucleic acid molecule that encodes a protein can result in expression or overexpression of that polypeptide in a transformed cell or transgenic plant. One or more of the proteins or fragments thereof encoded by nucleic acid molecules of the invention may be overexpressed in a transformed cell or transformed plant. Such expression or overexpression may be the result of transient or stable transfer of the exogenous genetic material.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α-tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of γ-tocopherols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant,

relative to an untransformed plant with a similar genetic background, an increased level of δ-tocopherols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of β-tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of tocotrienols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of α-tocotrienols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of γ-tocotrienols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of δ-tocotrienols.

In a preferred embodiment, reduction of the expression, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of β-tocotrienols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in combination with a nucleic acid molecule encoding any of the following enzymes: *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, tMT2, AANT1, slr1737, IDI, GGH* or a plant ortholog thereof, and an antisense construct for homogentisic acid dioxygenase in a plant, provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of total tocopherols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of plastoquinols.

In a preferred embodiment, expression or overexpression of a polypeptide of the present invention in a plant provides in that plant, relative to an untransformed plant with a similar genetic background, an increased level of total tocopherols.

In any of the embodiments described herein, an increase in γ-tocopherol, α-tocopherol, or both can lead to a decrease in the relative proportion of β-tocopherol, δ-tocopherol, or both. Similarly, an increase in γ-tocotienol, α-tocotrienol, or both can lead to a decrease in the relative proportion of β-tocotrienol, δ-tocotrienol, or both.

In another embodiment, expression, overexpression of a polypeptide of the present invention in a plant provides in that plant, or a tissue of that plant, relative to an untransformed plant or plant tissue, with a similar genetic background, an increased level of a tMT2 protein or fragment thereof.

In some embodiments, the levels of one or more products of the tocopherol biosynthesis pathway, including any one or more of tocopherols, α-tocopherols, γ-tocopherols, δ-tocopherols, β-tocopherols, tocotrienols, α-tocotrienols, γ-tocotrienols, δ-tocotrienols, β-tocotrienols are increased by greater than about 10%, or more preferably greater than about 25%, 35%, 50%, 75%, 80%, 90%, 100%, 150%, 200%, 1,000%, 2,000%, or 2,500%. The levels of products may be increased throughout an organism such as a plant or localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

In some embodiments, the levels of one or more products of the tocopherol biosynthesis pathway, including any one or more of tocopherols, α-tocopherols, γ-tocopherols, δ-tocopherols, β-tocopherols, tocotrienols, α-tocotrienols, γ-tocotrienols, δ-tocotrienols, β-tocotrienols are increased so that they constitute greater than about 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the total tocopherol content of the organism or tissue. The levels of products may be increased throughout an organism such as a plant or

localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed.

In a preferred embodiment, expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in the seed will result in an increase in γ-tocopherol levels due to the absence of significant levels of GMT activity in those tissues. In another preferred embodiment, expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in photosynthetic tissues will result in an increase in α-tocopherol due to the higher levels of GMT activity in those tissues relative to the same activity in seed tissue.

In another preferred embodiment, the expression of enzymes involved in tocopherol, tocotrienol or plastoquinol synthesis in the seed will result in an increase in the total tocopherol, tocotrienol or plastoquinol level in the plant.

In some embodiments, the levels of tocopherols or a species such as α-tocopherol may be altered. In some embodiments, the levels of tocotrienols may be altered. Such alteration can be compared to a plant with a similar background.

In another embodiment, either the α-tocopherol level, α-tocotrienol level, or both of plants that natively produce high levels of either α-tocopherol, α-tocotrienol or both (*e.g.*, sunflowers), can be increased by the introduction of a gene coding for a tMT2 enzyme.

In a preferred aspect, a similar genetic background is a background where the organisms being compared share about 50% or greater of their nuclear genetic material. In a more preferred aspect a similar genetic background is a background where the organisms being compared share about 75% or greater, even more preferably about 90% or greater of their nuclear genetic material. In another even more preferable aspect, a similar genetic background is a background where the organisms being compared are plants, and the plants are isogenic except for any genetic material originally introduced using plant transformation techniques.

In another preferred embodiment, reduction of the expression, expression, overexpression of a polypeptide of the present invention in a transformed plant may provide tolerance to a variety of stress, *e.g.* oxidative stress tolerance such as to oxygen or ozone, UV tolerance, cold tolerance, or fungal/microbial pathogen tolerance.

As used herein in a preferred aspect, a tolerance or resistance to stress is determined by the ability of a plant, when challenged by a stress such as cold to produce a plant having a higher yield than one without such tolerance or resistance to stress. In a particularly preferred aspect of the present invention, the tolerance or resistance to stress is measured relative to a plant with a similar genetic background to the tolerant or resistance plant except that the plant reduces the expression, expresses or over expresses a protein or fragment thereof of the present invention.

Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997)).

A construct or vector may include a plant promoter to express the polypeptide of choice. In a preferred embodiment, any nucleic acid molecules described herein can be operably linked to a promoter region which functions in a plant cell to cause the production of an mRNA molecule. For example, any promoter that functions in a plant cell to cause the production of an mRNA molecule, such as those promoters described herein, without limitation, can be used. In a preferred embodiment, the promoter is a plant promoter.

A number of promoters that are active in plant cells have been described in the literature. These include the nopaline synthase (NOS) promoter (Ebert *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:5745-5749 (1987)), the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al., Plant Mol. Biol. 9*:315-324 (1987)) and the CaMV 35S promoter (Odell *et al., Nature 313*:810-812 (1985)), the figwort mosaic virus 35S-promoter, the light-inducible promoter from the small subunit of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), the Adh promoter (Walker *et al., Proc. Natl. Acad. Sci. (U.S.A.) 84*:6624-6628 (1987)), the sucrose synthase promoter (Yang *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:4144-4148 (1990)), the R gene complex promoter (Chandler *et al., The Plant Cell 1*:1175-1183 (1989)) and the chlorophyll a/b binding protein gene promoter, *etc.* These promoters have been used to create DNA constructs that have been expressed in plants; *see, e.g.*, PCT publication WO 84/02913. The CaMV 35S promoters are preferred for use in plants. Promoters known or found to cause transcription of DNA in plant cells can be used in the invention.

For the purpose of expression in source tissues of the plant, such as the leaf, seed, root or stem, it is preferred that the promoters utilized have relatively high expression in these specific tissues. Tissue-specific expression of a protein of the present invention is a particularly preferred embodiment. For this purpose, one may choose from a number of promoters for genes with tissue- or cell-specific or enhanced expression. Examples of such promoters reported in the literature include the chloroplast glutamine synthetase GS2 promoter from pea (Edwards *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 87*:3459-3463 (1990)), the chloroplast fructose-1,6-biphosphatase (FBPase) promoter from wheat (Lloyd *et al.*, *Mol. Gen. Genet. 225*:209-216 (1991)), the nuclear photosynthetic ST-LS1 promoter from potato (Stockhaus *et al.*, *EMBO J. 8*:2445-2451 (1989)), the serine/threonine kinase (PAL) promoter and the glucoamylase (CHS) promoter from *Arabidopsis thaliana*. Also reported to be active in photosynthetically active tissues are the ribulose-1,5-bisphosphate carboxylase (RbcS) promoter from eastern larch (*Larix laricina*), the promoter for the *cab* gene, cab6, from pine (Yamamoto *et al.*, *Plant Cell Physiol. 35*:773-778 (1994)), the promoter for the Cab-1 gene from wheat (Fejes *et al.*, *Plant Mol. Biol. 15*:921-932 (1990)), the promoter for the CAB-1 gene from spinach (Lubberstedt *et al.*, *Plant Physiol. 104*:997-1006 (1994)), the promoter for the cab1R gene from rice (Luan *et al.*, *Plant Cell. 4*:971-981 (1992)), the pyruvate, orthophosphate dikinase (PPDK) promoter from corn (Matsuoka *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 90*:9586-9590 (1993)), the promoter for the tobacco Lhcb1*2 gene (Cerdan *et al.*, *Plant Mol. Biol. 33*:245-255 (1997)), the *Arabidopsis thaliana* SUC2 sucrose-H+ symporter promoter (Truernit *et al.*, *Planta. 196*:564-570 (1995)) and the promoter for the thylakoid membrane proteins from spinach (*psa*D, *psa*F, *psa*E, PC, FNR, *atp*C, *atp*D, *cab*, *rbc*S). Other promoters for the chlorophyll a/b-binding proteins may also be utilized in the invention, such as the promoters for *Lhc*B gene and *Psb*P gene from white mustard (*Sinapis alba*; Kretsch *et al.*, *Plant Mol. Biol. 28*:219-229 (1995)).

For the purpose of expression in sink tissues of the plant, such as the tuber of the potato plant, the fruit of tomato, or the seed of corn, wheat, rice and barley, it is preferred that the promoters utilized in the invention have relatively high expression in these specific tissues. A number of promoters for genes with tuber-specific or tuber-enhanced expression are known, including the class I patatin promoter (Bevan *et al.*, *EMBO J. 8*:1899-1906 (1986); Jefferson *et al.*, *Plant Mol. Biol. 14*:995-1006 (1990)), the promoter for the potato tuber ADPGPP genes, both the large and small subunits, the sucrose synthase promoter

(Salanoubat and Belliard, *Gene 60*:47-56 (1987), Salanoubat and Belliard, *Gene 84*:181-185 (1989)), the promoter for the major tuber proteins including the 22 kd protein complexes and protease inhibitors (Hannapel, *Plant Physiol. 101*:703-704 (1993)), the promoter for the granule-bound starch synthase gene (GBSS) (Visser *et al.*, *Plant Mol. Biol. 17*:691-699 (1991)) and other class I and II patatins promoters (Koster-Topfer *et al.*, *Mol. Gen. Genet. 219*:390-396 (1989); Mignery *et al.*, *Gene. 62*:27-44 (1988)).

Other promoters can also be used to express a polypeptide in specific tissues, such as seeds or fruits. Indeed, in a preferred embodiment, the promoter used is a seed specific promoter. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al., Seed Sci. Res.* 1:209:219 (1991)), phaseolin (Bustos, *et al., Plant Cell,* 1(9):839-853 (1989)), soybean trypsin inhibitor (Riggs, *et al., Plant Cell* 1(6):609-621 (1989)), ACP (Baerson, *et al., Plant Mol. Biol.*, 22(2):255-267 (1993)), stearoyl-ACP desaturase (Slocombe, *et al., Plant Physiol.* 104(4):167-176 (1994)), soybean a' subunit of b-conglycinin (soy 7s, (Chen *et al., Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))), and oleosin (see, for example, Hong, *et al., Plant Mol. Biol.*, 34(3):549-555 (1997)). Further examples include the promoter for β-conglycinin (Chen *et al., Dev. Genet. 10*:112-122 (1989)). Also included are the zeins, which are a group of storage proteins found in corn endosperm. Genomic clones for zein genes have been isolated (Pedersen *et al., Cell 29*:1015-1026 (1982), and Russell *et al., Transgenic Res. 6*(2):157-168) and the promoters from these clones, including the 15 kD, 16 kD, 19 kD, 22 kD, 27 kD and genes, could also be used. Other promoters known to function, for example, in corn include the promoters for the following genes: *waxy, Brittle, Shrunken 2*, Branching enzymes I and II, starch synthases, debranching enzymes, oleosins, glutelins and sucrose synthases. A particularly preferred promoter for corn endosperm expression is the promoter for the glutelin gene from rice, more particularly the Osgt-1 promoter (Zheng *et al., Mol. Cell Biol. 13*:5829-5842 (1993)). Examples of promoters suitable for expression in wheat include those promoters for the ADPglucose pyrosynthase (ADPGPP) subunits, the granule bound and other starch synthase, the branching and debranching enzymes, the embryogenesis-abundant proteins, the gliadins and the glutenins. Examples of such promoters in rice include those promoters for the ADPGPP subunits, the granule bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases and the glutelins. A particularly preferred promoter is the promoter for rice glutelin, Osgt-1. Examples of such promoters for barley include those for the ADPGPP subunits, the granule

bound and other starch synthase, the branching enzymes, the debranching enzymes, sucrose synthases, the hordeins, the embryo globulins and the aleurone specific proteins. A preferred promoter for expression in the seed is a napin promoter. Another preferred promoter for expression is an Arcelin5 promoter.

Root specific promoters may also be used. An example of such a promoter is the promoter for the acid chitinase gene (Samac *et al., Plant Mol. Biol. 25*:587-596 (1994)). Expression in root tissue could also be accomplished by utilizing the root specific subdomains of the CaMV35S promoter that have been identified (Lam *et al., Proc. Natl. Acad. Sci. (U.S.A.) 86*:7890-7894 (1989)). Other root cell specific promoters include those reported by Conkling *et al.* (Conkling *et al., Plant Physiol. 93*:1203-1211 (1990)).

Additional promoters that may be utilized are described, for example, in U.S. Patents 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used (Fromm *et al., The Plant Cell 1*:977-984 (1989)).

Constructs or vectors may also include, with the coding region of interest, a nucleic acid sequence that acts, in whole or in part, to terminate transcription of that region. A number of such sequences have been isolated, including the Tr7 3' sequence and the NOS 3' sequence (Ingelbrecht *et al., The Plant Cell 1*:671-680 (1989); Bevan *et al., Nucleic Acids Res. 11*:369-385 (1983)). Regulatory transcript termination regions can be provided in plant expression constructs of this invention as well. Transcript termination regions can be provided by the DNA sequence encoding the gene of interest or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region that is naturally associated with the transcript initiation region. The skilled artisan will recognize that any convenient transcript termination region that is capable of terminating transcription in a plant cell can be employed in the constructs of the present invention.

A vector or construct may also include regulatory elements. Examples of such include the Adh intron 1 (Callis *et al., Genes and Develop. 1*:1183-1200 (1987)), the sucrose synthase intron (Vasil *et al., Plant Physiol. 91*:1575-1579 (1989)) and the TMV omega element (Gallie *et al., The Plant Cell 1*:301-311 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to: a *neo* gene (Potrykus *et al.*, *Mol. Gen. Genet. 199*:183-188 (1985)), which codes for kanamycin resistance and can be selected for using kanamycin, RptII, G418, hpt *etc.*; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology 6*:915-922 (1988); Reynaerts *et al.*, Selectable and Screenable Markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988); Reynaerts *et al.*, Selectable and screenable markers. In Gelvin and Schilperoort. Plant Molecular Biology Manual, Kluwer, Dordrecht (1988)), *aad*A (Jones *et al.*, *Mol. Gen. Genet.* (1987)),) which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem. 263*:6310-6314 (1988)); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204 (Sept. 11, 1985)), ALS (D'Halluin *et al.*, Bio/Technology 10:309-314 (1992)), and a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem. 263*:12500-12508 (1988)).

A vector or construct may also include a transit peptide. Incorporation of a suitable chloroplast transit peptide may also be employed (European Patent Application Publication Number 0218571). Translational enhancers may also be incorporated as part of the vector DNA. DNA constructs could contain one or more 5' non-translated leader sequences, which may serve to enhance expression of the gene products from the resulting mRNA transcripts. Such sequences may be derived from the promoter selected to express the gene or can be specifically modified to increase translation of the mRNA. Such regions may also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. For a review of optimizing expression of transgenes, see Koziel *et al.*, *Plant Mol. Biol. 32*:393-405 (1996). A preferred transit peptide is CTP1.

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include: a β-glucuronidase or *uid*A gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol., Rep. 5*:387-405 (1987); Jefferson *et al.*, *EMBO J. 6*:3901-3907 (1987)); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, Stadler Symposium *11*:263-282 (1988)); a β-lactamase gene (Sutcliffe *et*

al., Proc. Natl. Acad. Sci. (U.S.A.) 75:3737-3741 (1978)), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al., Science 234*:856-859 (1986)); a *xyl*E gene (Zukowsky *et al., Proc. Natl. Acad. Sci. (U.S.A.) 80*:1101-1105 (1983)) which encodes a catechol dioxygenase that can convert chromogenic catechols; an α-amylase gene (Ikatu *et al., Bio/Technol. 8*:241-242 (1990)); a tyrosinase gene (Katz *et al., J. Gen. Microbiol. 129*:2703-2714 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; an α-galactosidase, which will turn a chromogenic α-galactose substrate.

Included within the terms "selectable or screenable marker genes" are also genes that encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes that can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins that are detectable, (*e.g.*, by ELISA), small active enzymes that are detectable in extracellular solution (*e.g.*, α-amylase, β-lactamase, phosphinothricin transferase), or proteins that are inserted or trapped in the cell wall (such as proteins that include a leader sequence such as that found in the expression unit of extension or tobacco PR-S). Other possible selectable and/or screenable marker genes will be apparent to those of skill in the art.

There are many methods for introducing transforming nucleic acid molecules into plant cells. Suitable methods are believed to include virtually any method by which nucleic acid molecules may be introduced into a cell, such as by *Agrobacterium* infection or direct delivery of nucleic acid molecules such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, and the like. (Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol. 42*:205-225 (1991); Vasil, *Plant Mol. Biol. 25*:925-937 (1994)). For example, electroporation has been used to transform corn protoplasts (Fromm *et al., Nature 312*:791-793 (1986)).

Other vector systems suitable for introducing transforming DNA into a host plant cell include but are not limited to binary artificial chromosome (BIBAC) vectors (Hamilton *et al., Gene 200*:107-116 (1997)); and transfection with RNA viral vectors (Della-Cioppa *et al., Ann. N.Y. Acad. Sci.* (1996), 792 (Engineering Plants for Commercial Products and

Applications), 57-61). Additional vector systems also include plant selectable YAC vectors such as those described in Mullen *et al.*, *Molecular Breeding 4*:449-457 (1988).

Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun. 107*:584-587 (1982); Fromm *et al., Proc. Natl. Acad. Sci. (U.S.A.) 82*:5824-5828 (1985); U.S. Patent 5,384,253); the gene gun (Johnston and Tang, *Methods Cell Biol. 43*:353-365 (1994)); and vacuum infiltration (Bechtold *et al., C.R. Acad. Sci. Paris, Life Sci. 316*:1194-1199. (1993)); (3) viral vectors (Clapp, *Clin. Perinatol. 20*:155-168 (1993); Lu *et al., J. Exp. Med. 178*:2089-2096 (1993); Eglitis and Anderson, *Biotechniques 6*:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al., Hum. Gen. Ther. 3*:147-154 (1992), Wagner *et al., Proc. Natl. Acad. Sci. (USA) 89*:6099-6103 (1992)).

Acceleration methods that may be used include, for example, microprojectile bombardment and the like. One example of a method for delivering transforming nucleic acid molecules into plant cells is microprojectile bombardment. This method has been reviewed by Yang and Christou (eds.), *Particle Bombardment Technology for Gene Transfer*, Oxford Press, Oxford, England (1994)). Non-biological particles (microprojectiles) may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum and the like.

A particular advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly transforming monocots, is that neither the isolation of protoplasts (Cristou *et al., Plant Physiol. 87*:671-674 (1988)) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into corn cells by acceleration is a biolistics α-particle delivery system, which can be used to propel particles coated with DNA through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. Gordon-Kamm *et al.*, describes the basic procedure for coating tungsten particles with DNA (Gordon-Kamm *et al., Plant Cell 2*:603-618 (1990)). The screen disperses the tungsten nucleic acid particles so that they are not delivered to the recipient cells in large aggregates. A particle delivery system suitable for use with the invention is

the helium acceleration PDS-1000/He gun, which is available from Bio-Rad Laboratories (Bio-Rad, Hercules, California)(Sanford *et al.*, *Technique 3*:3-16 (1991)).

For the bombardment, cells in suspension may be concentrated on filters. Filters containing the cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the gun and the cells to be bombarded.

Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain 1000 or more loci of cells transiently expressing a marker gene. The number of cells in a focus that express the exogenous gene product 48 hours post-bombardment often ranges from one to ten, and average one to three.

In bombardment transformation, one may optimize the pre-bombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

In another alternative embodiment, plastids can be stably transformed. Methods disclosed for plastid transformation in higher plants include the particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination (Svab *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 87*:8526-8530 (1990); Svab and Maliga, *Proc. Natl. Acad. Sci. (U.S.A.) 90*:913-917 (1993); Staub and Maliga, *EMBO J. 12*:601-606 (1993); U.S. Patents 5, 451,513 and 5,545,818).

Accordingly, it is contemplated that one may wish to adjust various aspects of the bombardment parameters in small scale studies to fully optimize the conditions. One may

particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance and helium pressure. One may also minimize the trauma reduction factors by modifying conditions that influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example the methods described by Fraley *et al.*, *Bio/Technology 3*:629-635 (1985) and Rogers *et al.*, *Methods Enzymol. 153*:253-277 (1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, *Mol. Gen. Genet. 205*:34 (1986)).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985)). Moreover, technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes (Rogers *et al.*, *Methods Enzymol. 153*:253-277 (1987)). In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. More preferred is a transgenic plant that is

homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant, transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for the gene of interest.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation and combinations of these treatments (*See, for example*, Potrykus *et al., Mol. Gen. Genet. 205*:193-200 (1986); Lorz *et al., Mol. Gen. Genet. 199*:178 (1985); Fromm *et al., Nature 319*:791 (1986); Uchimiya *et al., Mol. Gen. Genet. 204*:204 (1986); Marcotte *et al., Nature 335*:454-457 (1988)).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (Fujimura *et al., Plant Tissue Culture Letters 2*:74 (1985); Toriyama *et al., Theor. Appl. Genet. 205*:34 (1986); Yamada *et al., Plant Cell Rep. 4*:85 (1986); Abdullah *et al., Biotechnology 4*:1087 (1986)).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, *Biotechnology 6*:397 (1988)). In addition, "particle gun" or high-velocity microprojectile technology can be utilized (Vasil *et al., Bio/Technology 10*:667 (1992)).

Using the latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al., Nature 328*:70 (1987); Klein *et al., Proc. Natl. Acad. Sci. (U.S.A.) 85*:8502-8505 (1988); McCabe *et al., Bio/Technology 6:923* (1988)). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen (Hess *et al.*, *Intern Rev. Cytol. 107*:367 (1987); Luo *et al.*, *Plant Mol Biol. Reporter 6*:165 (1988)), by direct injection of DNA into reproductive organs of a plant (Pena *et al.*, *Nature 325*:274 (1987)), or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos (Neuhaus *et al.*, *Theor. Appl. Genet. 75*:30 (1987)).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA, (1988)). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent 5,004,863; U.S. Patent 5,159,135; U.S. Patent 5,518,908); soybean (U.S. Patent 5,569,834; U.S. Patent 5,416,011; McCabe *et al.*, *Biotechnology 6*:923 (1988); Christou *et al.*, *Plant Physiol. 87*:671-674 (1988)); *Brassica* (U.S. Patent 5,463,174); peanut (Cheng *et al.*, *Plant Cell Rep. 15*:653-657 (1996), McKently *et al.*, *Plant Cell Rep. 14*:699-703 (1995)); papaya; pea (Grant *et al.*, *Plant Cell Rep. 15*:254-258 (1995)); and *Arabidopsis thaliana* (Bechtold *et al.*, *C.R. Acad. Sci. Paris, Life Sci. 316*:1194-1199 (1993)). The latter method for

transforming *Arabidopsis thaliana* is commonly called "dipping" or vacuum infiltration or germplasm transformation.

Transformation of monocotyledons using electroporation, particle bombardment and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier *et al., Proc. Natl. Acad. Sci. (USA) 84*:5354 (1987)); barley (Wan and Lemaux, *Plant Physiol 104*:37 (1994)); corn (Rhodes *et al., Science 240*:204 (1988); Gordon-Kamm *et al., Plant Cell* 2:603-618 (1990); Fromm *et al., Bio/Technology 8*:833 (1990); Koziel *et al., Bio/Technology 11*:194 (1993); Armstrong *et al., Crop Science 35*:550-557 (1995)); oat (Somers *et al., Bio/Technology 10*:1589 (1992)); orchard grass (Horn *et al., Plant Cell Rep. 7*:469 (1988)); rice (Toriyama *et al., Theor Appl. Genet. 205*:34 (1986); Part *et al., Plant Mol. Biol. 32*:1135-1148 (1996); Abedinia *et al., Aust. J. Plant Physiol. 24*:133-141 (1997); Zhang and Wu, *Theor. Appl. Genet. 76*:835 (1988); Zhang *et al., Plant Cell Rep. 7*:379 (1988); Battraw and Hall, *Plant Sci. 86*:191-202 (1992); Christou *et al., Bio/Technology 9*:957 (1991)); rye (De la Pena *et al., Nature 325*:274 (1987)); sugarcane (Bower and Birch, *Plant J. 2*:409 (1992)); tall fescue (Wang *et al., Bio/Technology 10*:691 (1992)) and wheat (Vasil *et al., Bio/Technology 10*:667 (1992); U.S. Patent 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al., Nature 335*:454-457 (1988); Marcotte *et al., Plant Cell 1*:523-532 (1989); McCarty *et al., Cell 66*:895-905 (1991); Hattori *et al., Genes Dev. 6*:609-618 (1992); Goff *et al., EMBO J. 9*:2517-2522 (1990)). Transient expression systems may be used to functionally dissect gene constructs (*see generally*, Mailga *et al., Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995)).

Any of the nucleic acid molecules of the invention may be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers, *etc.* Further, any of the nucleic acid molecules of the invention may be introduced into a plant cell in a manner that allows for expression or overexpression of the protein or fragment thereof encoded by the nucleic acid molecule.

Cosuppression is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense

construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene (Napoli *et al., Plant Cell 2*:279-289 (1990); van der Krol *et al., Plant Cell 2*:291-299 (1990)). Cosuppression may result from stable transformation with a single copy nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Prolls and Meyer, *Plant J. 2*:465-475 (1992)) or with multiple copies of a nucleic acid molecule that is homologous to a nucleic acid sequence found with the cell (Mittlesten *et al., Mol. Gen. Genet. 244*:325-330 (1994)). Genes, even though different, linked to homologous promoters may result in the cosuppression of the linked genes (Vaucheret, *C.R. Acad. Sci. III 316*:1471-1483 (1993); Flavell, *Proc. Natl. Acad. Sci. (U.S.A.) 91*:3490-3496 (1994)); van Blokland *et al., Plant J. 6*:861-877 (1994); Jorgensen, *Trends Biotechnol. 8*:340-344 (1990); Meins and Kunz, In: *Gene Inactivation and Homologous Recombination in Plants*, Paszkowski (ed.), pp. 335-348, Kluwer Academic, Netherlands (1994)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the cosuppression of an endogenous protein.

Antisense approaches are a way of preventing or reducing gene function by targeting the genetic material (Mol *et al., FEBS Lett. 268*:427-430 (1990)). The objective of the antisense approach is to use a sequence complementary to the target gene to block its expression and create a mutant cell line or organism in which the level of a single chosen protein is selectively reduced or abolished. Antisense techniques have several advantages over other 'reverse genetic' approaches. The site of inactivation and its developmental effect can be manipulated by the choice of promoter for antisense genes or by the timing of external application or microinjection. Antisense can manipulate its specificity by selecting either unique regions of the target gene or regions where it shares homology to other related genes (Hiatt *et al.,* In: *Genetic Engineering,* Setlow (ed.), Vol. 11, New York: Plenum 49-63 (1989)).

Antisense RNA techniques involve introduction of RNA that is complementary to the target mRNA into cells, which results in specific RNA:RNA duplexes being formed by base pairing between the antisense substrate and the target mRNA (Green *et al., Annu. Rev. Biochem. 55*:569-597 (1986)). Under one embodiment, the process involves the introduction and expression of an antisense gene sequence. Such a sequence is one in which part or all of the normal gene sequences are placed under a promoter in inverted

orientation so that the 'wrong' or complementary strand is transcribed into a noncoding antisense RNA that hybridizes with the target mRNA and interferes with its expression (Takayama and Inouye, *Crit. Rev. Biochem. Mol. Biol. 25*:155-184 (1990)). An antisense vector is constructed by standard procedures and introduced into cells by transformation, transfection, electroporation, microinjection, infection, *etc.* The type of transformation and choice of vector will determine whether expression is transient or stable. The promoter used for the antisense gene may influence the level, timing, tissue, specificity, or inducibility of the antisense inhibition.

It is understood that the activity of a protein in a plant cell may be reduced or depressed by growing a transformed plant cell containing a nucleic acid molecule whose non-transcribed strand encodes a protein or fragment thereof. A preferred protein whose activity can be reduced or depressed, by any method, is tMT2. In such an embodiment of the invention, it is preferred that the concentration of δ-tocopherol or δ-tocotrienol is increased. Another preferred protein whose activity can be reduced or depressed, by any method, is homogentisic acid dioxygenase.

Posttranscriptional gene silencing (PTGS) can result in virus immunity or gene silencing in plants. PTGS is induced by dsRNA and is mediated by an RNA-dependent RNA polymerase, present in the cytoplasm, which requires a dsRNA template. The dsRNA is formed by hybridization of complementary transgene mRNAs or complementary regions of the same transcript. Duplex formation can be accomplished by using transcripts from one sense gene and one antisense gene colocated in the plant genome, a single transcript that has self-complementarity, or sense and antisense transcripts from genes brought together by crossing. The dsRNA-dependent RNA polymerase makes a complementary strand from the transgene mRNA and RNAse molecules attach to this complementary strand (cRNA). These cRNA-RNase molecules hybridize to the endogene mRNA and cleave the single-stranded RNA adjacent to the hybrid. The cleaved single-stranded RNAs are further degraded by other host RNases because one will lack a capped 5' end and the other will lack a poly(A) tail (Waterhouse *et al.*, *PNAS 95*:13959-13964 (1998)).

It is understood that one or more of the nucleic acids of the invention may be introduced into a plant cell and transcribed using an appropriate promoter with such transcription resulting in the postranscriptional gene silencing of an endogenous transcript.

Antibodies have been expressed in plants (Hiatt *et al.*, *Nature 342*:76-78 (1989); Conrad and Fielder, *Plant Mol. Biol. 26*:1023-1030 (1994)). Cytoplasmic expression of a scFv (single-chain Fv antibody) has been reported to delay infection by artichoke mottled crinkle virus. Transgenic plants that express antibodies directed against endogenous proteins may exhibit a physiological effect (Philips *et al.*, *EMBO J. 16*:4489-4496 (1997); Marion-Poll, *Trends in Plant Science 2*:447-448 (1997)). For example, expressed anti-abscisic antibodies have been reported to result in a general perturbation of seed development (Philips *et al.*, *EMBO J. 16*:4489-4496 (1997)).

Antibodies that are catalytic may also be expressed in plants (abzymes). The principle behind abzymes is that since antibodies may be raised against many molecules, this recognition ability can be directed toward generating antibodies that bind transition states to force a chemical reaction forward (Persidas, *Nature Biotechnology 15*:1313-1315 (1997); Baca *et al.*, *Ann. Rev. Biophys. Biomol. Struct. 26*:461-493 (1997)). The catalytic abilities of abzymes may be enhanced by site directed mutagenesis. Examples of abzymes are, for example, set forth in U.S. Patent: 5,658,753; U.S. Patent 5,632,990; U.S. Patent 5,631,137; U.S. Patent 5,602,015; U.S. Patent 5,559,538; U.S. Patent 5,576,174; U.S. Patent 5,500,358; U.S. Patent 5,318,897; U.S. Patent 5,298,409; U.S. Patent 5,258,289 and U.S. Patent 5,194,585.

It is understood that any of the antibodies of the invention may be expressed in plants and that such expression can result in a physiological effect. It is also understood that any of the expressed antibodies may be catalytic.

The present invention also provides for parts of the plants, particularly reproductive or storage parts, of the present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred embodiment of the present invention, the plant part is a seed. In one embodiment the seed is a constituent of animal feed.

In another embodiment, the plant part is a fruit, more preferably a fruit with enhanced shelf life. In another preferred embodiment, the fruit has increased levels of a tocopherol. In another preferred embodiment, the fruit has increased levels of a tocotrienol.

The present invention also provides a container of over about 10,000, more preferably about 20,000, and even more preferably about 40,000 seeds where over about

10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

The present invention also provides a container of over about 10 kg, more preferably about 25 kg, and even more preferably about 50 kg seeds where over about 10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

Any of the plants or parts thereof of the present invention may be processed to produce a feed, meal, protein, or oil preparation, including oil preparations high in total tocopherol content and oil preparations high in any one or more of each tocopherol component listed herein. A particularly preferred plant part for this purpose is a seed. In a preferred embodiment the feed, meal, protein or oil preparation is designed for livestock animals or humans, or both. Methods to produce feed, meal, protein and oil preparations are known in the art. See, for example, U.S. Patents 4,957,748, 5,100,679, 5,219,596, 5,936,069, 6,005,076, 6,146,669, and 6,156,227. In a preferred embodiment, the protein preparation is a high protein preparation. Such a high protein preparation preferably has a protein content of greater than about 5% w/v, more preferably about 10% w/v, and even more preferably about 15% w/v. In a preferred oil preparation, the oil preparation is a high oil preparation with an oil content derived from a plant or part thereof of the present invention of greater than about 5% w/v, more preferably about 10% w/v, and even more preferably about 15% w/v. In a preferred embodiment the oil preparation is a liquid and of a volume greater than about 1, 5, 10 or 50 liters. The present invention provides for oil produced from plants of the present invention or generated by a method of the present invention. Such an oil may exhibit enhanced oxidative stability. Also, such oil may be a minor or major component of any resultant product. Moreover, such oil may be blended with other oils. In a preferred embodiment, the oil produced from plants of the present invention or generated by a method of the present invention constitutes greater than about 0.5%, 1%, 5%, 10%, 25%, 50%, 75% or 90% by volume or weight of the oil component of any product. In another embodiment, the oil preparation may be blended and can constitute greater than about 10%, 25%, 35%, 50% or 75% of the blend by volume. Oil produced from a plant of the present invention can be admixed with one or more organic solvents or petroleum distillates.

Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability

of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F_1 hybrid cultivar, pureline cultivar, etc). Selected, non-limiting approaches, for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program. Factors such as, for example, emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering, seed set, seed size, seed density, standability, and threshability etc. will generally dictate the choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection. In a preferred embodiment a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates for new commercial cultivars; those still deficient in traits may be used as parents to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated selfing and selection, producing many new genetic combinations.

The development of new cultivars requires the development and selection of varieties, the crossing of these varieties and the selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as pod color, flower color, seed yield, pubescence color, or herbicide resistance, which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. New cultivars can be evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1. A F_2 population is produced by selfing one or several F_1's. Selection of the best individuals from the best families is carried out. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (*i.e.*, F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F_2 to the

desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

In a multiple-seed procedure, breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seed of a population each generation of inbreeding.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (*e.g.* Fehr, *Principles of Cultivar Development* Vol. 1, pp. 2-3 (1987))).

A transgenic plant of the present invention may also be reproduced using apomixis. Apomixis is a genetically controlled method of reproduction in plants where the embryo is formed without union of an egg and a sperm. There are three basic types of apomictic reproduction: 1) apospory where the embryo develops from a chromosomally unreduced egg in an embryo sac derived from the nucleus, 2) diplospory where the embryo develops from an unreduced egg in an embryo sac derived from the megaspore mother cell, and 3) adventitious embryo where the embryo develops directly from a somatic cell. In most forms of apomixis, pseudogamy or fertilization of the polar nuclei to produce endosperm is necessary for seed viability. In apospory, a nurse cultivar can be used as a pollen source for endosperm formation in seeds. The nurse cultivar does not affect the genetics of the aposporous apomictic cultivar since the unreduced egg of the cultivar develops parthenogenetically, but makes possible endosperm production. Apomixis is economically important, especially in transgenic plants, because it causes any genotype, no matter how heterozygous, to breed true. Thus, with apomictic reproduction, heterozygous transgenic plants can maintain their genetic fidelity throughout repeated life cycles. Methods for the production of apomictic plants are known in the art. *See*, U.S. Patent 5,811,636.

OTHER ORGANISMS

A nucleic acid of the present invention may be introduced into any cell or organism such as a mammalian cell, mammal, fish cell, fish, bird cell, bird, algae cell, algae, fungal cell, fungi, or bacterial cell. A protein of the present invention may be produced in an appropriate cell or organism. Preferred host and transformants include: fungal cells such as *Aspergillus*, yeasts, mammals, particularly bovine and porcine, insects, bacteria, and algae. Particularly preferred bacteria are *Agrobacteruim tumefaciens* and *E. coli.*

Methods to transform such cells or organisms are known in the art (EP 0 238 023; Yelton *et al., Proc. Natl. Acad. Sci. (U.S.A.), 81*:1470-1474 (1984); Malardier *et al., Gene, 78*:147-156 (1989); Becker and Guarente, In: Abelson and Simon (eds.), *Guide to Yeast Genetics and Molecular Biology, Method Enzymol.*, Vol. 194, pp. 182-187, Academic Press, Inc., New York; Ito *et al., J. Bacteriology, 153*:163 (1983) Hinnen *et al., Proc. Natl. Acad. Sci. (U.S.A.), 75*:1920 (1978); Bennett and LaSure (eds.), *More Gene Manipualtionins in fungi*, Academic Press, CA (1991)). Methods to produce proteins of the present invention are also known (Kudla *et al., EMBO, 9*:1355-1364 (1990); Jarai and Buxton, *Current Genetics, 26*:2238-2244 (1994); Verdier, *Yeast, 6*:271-297 (1990; MacKenzie *et al., Journal of Gen. Microbiol., 139*:2295-2307 (1993); Hartl *et al., TIBS, 19*:20-25 (1994); Bergenron *et al., TIBS, 19*:124-128 (1994); Demolder *et al., J. Biotechnology, 32*:179-189 (1994); Craig, *Science, 260*:1902-1903 (1993); Gething and Sambrook, *Nature, 355*:33-45 (1992); Puig and Gilbert, J. *Biol. Chem., 269*:7764-7771 (1994); Wang and Tsou, *FASEB Journal, 7*:1515-1517 (1993); Robinson *et al., Bio/Technology, 1*:381-384 (1994); Enderlin and Ogrydziak, *Yeast, 10*:67-79 (1994); Fuller *et al., Proc. Natl. Acad. Sci. (U.S.A.), 86*:1434-1438 (1989); Julius *et al., Cell, 37*:1075-1089 (1984); Julius *et al., Cell 32*:839-852 (1983).

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of α-tocopherols.

In a preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ-tocopherols.

In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of α-tocotrienols.

In another preferred embodiment, overexpression of a protein or fragment thereof of the present invention in a cell or organism provides in that cell or organism, relative to an untransformed cell or organism with a similar genetic background, an increased level of γ-tocotrienols.

ANTIBODIES

One aspect of the invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the invention and their homologs, fusions or fragments. In a particularly preferred embodiment, the antibody specifically binds to a protein having the amino acid sequence set forth in SEQ ID NOs: 16 through 38 or fragments thereof. In another embodiment, the antibody specifically binds to a fusion protein comprising an amino acid sequence selected from the amino acid sequence set forth in SEQ ID NOs: 16 through 38 or fragments thereof. Antibodies of the invention may be used to quantitatively or qualitatively detect the protein or peptide molecules of the invention, or to detect post translational modifications of the proteins. As used herein, an antibody or peptide is said to "specifically bind" to a protein or peptide molecule of the invention if such binding is not competitively inhibited by the presence of non-related molecules.

Nucleic acid molecules that encode all or part of the protein of the invention can be expressed, via recombinant means, to yield protein or peptides that can in turn be used to elicit antibodies that are capable of binding the expressed protein or peptide. Such antibodies may be used in immunoassays for that protein. Such protein-encoding molecules, or their fragments may be a "fusion" molecule (*i.e.*, a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced. It is understood

that any of the nucleic acid molecules of the invention may be expressed, via recombinant means, to yield proteins or peptides encoded by these nucleic acid molecules.

The antibodies that specifically bind proteins and protein fragments of the invention may be polyclonal or monoclonal and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins fragments (such as (F(ab'), F(ab')$_2$), or single-chain immunoglobulins producible, for example, via recombinant means. It is understood that practitioners are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation and isolation of antibodies (*see*, for example, Harlow and Lane, In: *Antibodies: A Laboratory Manual,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1988)).

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind the protein or peptide molecules of the invention permits the identification of mimetic compounds derived from those molecules. These mimetic compounds may contain a fragment of the protein or peptide or merely a structurally similar region and nonetheless exhibits an ability to specifically bind to antibodies directed against that compound.

EXEMPLARY USES

Nucleic acid molecules and fragments thereof of the invention may be employed to obtain other nucleic acid molecules from the same species (nucleic acid molecules from corn may be utilized to obtain other nucleic acid molecules from corn). Such nucleic acid molecules include the nucleic acid molecules that encode the complete coding sequence of a protein and promoters and flanking sequences of such molecules. In addition, such nucleic acid molecules include nucleic acid molecules that encode for other isozymes or gene family members. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries. Methods for forming such libraries are well known in the art.

Nucleic acid molecules and fragments thereof of the invention may also be employed to obtain nucleic acid homologs. Such homologs include the nucleic acid molecules of plants and other organisms, including bacteria and fungi, including the

nucleic acid molecules that encode, in whole or in part, protein homologues of other plant species or other organisms, sequences of genetic elements, such as promoters and transcriptional regulatory elements. Such molecules can be readily obtained by using the above-described nucleic acid molecules or fragments thereof to screen cDNA or genomic libraries obtained from such plant species. Methods for forming such libraries are well known in the art. Such homolog molecules may differ in their nucleotide sequences from those found in one or more of SEQ ID NOs: 1 through 15, and complements thereof because complete complementarity is not needed for stable hybridization. The nucleic acid molecules of the invention therefore also include molecules that, although capable of specifically hybridizing with the nucleic acid molecules may lack "complete complementarity."

Any of a variety of methods may be used to obtain one or more of the above-described nucleic acid molecules (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 83*:4143-4146 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:5507-5511 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:1028-1032 (1988); Holt *et al.*, *Molec. Cell. Biol. 8*:963-973 (1988); Gerwirtz *et al.*, *Science 242*:1303-1306 (1988); Anfossi *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 86*:3379-3383 (1989); Becker *et al.*, *EMBO J. 8*:3685-3691 (1989)). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed nucleic acid molecules may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986); Erlich *et al.*, European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; Mullis, European Patent 201,184; Mullis *et al.*, U.S. Patent 4,683,202; Erlich, U.S. Patent 4,582,788; and Saiki *et al.*, U.S. Patent 4,683,194) to amplify and obtain any desired nucleic acid molecule or fragment.

Promoter sequences and other genetic elements, including but not limited to transcriptional regulatory flanking sequences, associated with one or more of the disclosed nucleic acid sequences can also be obtained using the disclosed nucleic acid sequence provided herein. In one embodiment, such sequences are obtained by incubating nucleic acid molecules of the present invention with members of genomic libraries and recovering clones that hybridize to such nucleic acid molecules thereof. In a second embodiment, methods of "chromosome walking," or inverse PCR may be used to obtain such sequences (Frohman *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.) 85*:8998-9002 (1988); Ohara *et al.*, *Proc.*

Natl. Acad. Sci. (U.S.A.) 86:5673-5677 (1989); Pang *et al., Biotechniques 22:*1046-1048 (1977); Huang *et al., Methods Mol. Biol. 69:*89-96 (1997); Huang *et al., Method Mol. Biol. 67:*287-294 (1997); Benkel *et al., Genet. Anal. 13:*123-127 (1996); Hartl *et al., Methods Mol. Biol. 58:*293-301 (1996)). The term "chromosome walking" means a process of extending a genetic map by successive hybridization steps.

The nucleic acid molecules of the invention may be used to isolate promoters of cell enhanced, cell specific, tissue enhanced, tissue specific, developmentally or environmentally regulated expression profiles. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See, for example, Birren *et al., Genome Analysis: Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Promoters obtained utilizing the nucleic acid molecules of the invention could also be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhancer sequences. Such genetic elements could be used to enhance gene expression of new and existing traits for crop improvement.

Another subset of the nucleic acid molecules of the invention includes nucleic acid molecules that are markers. The markers can be used in a number of conventional ways in the field of molecular genetics. Such markers include nucleic acid molecules SEQ ID NOs: 1 through 15, complements thereof, and fragments of either that can act as markers and other nucleic acid molecules of the present invention that can act as markers.

Genetic markers of the invention include "dominant" or "codominant" markers. "Codominant markers" reveal the presence of two or more alleles (two per diploid individual) at a locus. "Dominant markers" reveal the presence of only a single allele per locus. The presence of the dominant marker phenotype (e.g., a band of DNA) is an indication that one allele is in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (*e.g.*, absence of a DNA band) is merely evidence that "some other" undefined allele is present. In the case of populations where individuals are predominantly homozygous and loci are predominately dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multi-allelic, codominant markers often become more informative of the

genotype than dominant markers. Marker molecules can be, for example, capable of detecting polymorphisms such as single nucleotide polymorphisms (SNPs).

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, *Ann. Rev. Biochem. 55*:831-854 (1986)). A "polymorphism" is a variation or difference in the sequence of the gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a population may have the original sequence (i.e., the original "allele") whereas other members may have the variant sequence (i.e., the variant "allele"). In the simplest case, only one variant sequence may exist and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles and the polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity analysis (Weber, U.S. Patent 5,075,217; Armour *et al., FEBS Lett. 307*:113-115 (1992); Jones *et al., Eur. J. Haematol. 39*:144-147 (1987); Horn *et al.*, PCT Patent Application WO91/14003; Jeffreys, European Patent Application 370,719; Jeffreys, U.S. Patent 5,175,082; Jeffreys *et al., Amer. J. Hum. Genet. 39*:11-24 (1986); Jeffreys *et al., Nature 316*:76-79 (1985); Gray *et al., Proc. R. Acad. Soc. Lond. 243*:241-253 (1991); Moore *et al., Genomics 10*:654-660 (1991); Jeffreys *et al., Anim. Genet. 18*:1-15 (1987); Hillel *et al., Anim. Genet. 20*:145-155 (1989); Hillel *et al., Genet. 124*:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and

sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s) and more preferably within 100kb of the polymorphism(s) and most preferably within 10kb of the polymorphism(s) can be employed.

The identification of a polymorphism can be determined in a variety of ways. By correlating the presence or absence of it in a plant with the presence or absence of a phenotype, it is possible to predict the phenotype of that plant. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, organisms that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs") (Glassberg, UK Patent Application 2135774; Skolnick *et al., Cytogen. Cell Genet. 32*:58-67 (1982); Botstein *et al., Ann. J. Hum. Genet. 32*:314-331 (1980); Fischer *et al.*, (PCT Application WO90/13668; Uhlen, PCT Application WO90/11369).

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996)); Orita *et al., Genomics 5*:874-879 (1989)). A number of protocols have been described for SSCP including, but not limited to, Lee *et al., Anal. Biochem. 205*:289-293 (1992); Suzuki *et al., Anal. Biochem. 192*:82-84 (1991); Lo *et al., Nucleic Acids Research 20*:1005-1009 (1992); Sarkar *et al., Genomics 13*:441-443 (1992). It is understood that one or more of the nucleic acids of the invention, may be utilized as markers or probes to detect polymorphisms by SSCP analysis.

Polymorphisms may also be found using a DNA fingerprinting technique called amplified fragment length polymorphism (AFLP), which is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA to profile that DNA (Vos *et al., Nucleic Acids Res. 23*:4407-4414 (1995)). This method allows for the

specific co-amplification of high numbers of restriction fragments, which can be visualized by PCR without knowledge of the nucleic acid sequence. It is understood that one or more of the nucleic acids of the invention may be utilized as markers or probes to detect polymorphisms by AFLP analysis or for fingerprinting RNA.

Polymorphisms may also be found using random amplified polymorphic DNA (RAPD) (Williams *et al., Nucl. Acids Res. 18*:6531-6535 (1990)) and cleaveable amplified polymorphic sequences (CAPS) (Lyamichev *et al., Science 260*:778-783 (1993)). It is understood that one or more of the nucleic acid molecules of the invention, may be utilized as markers or probes to detect polymorphisms by RAPD or CAPS analysis.

Single Nucleotide Polymorphisms (SNPs) generally occur at greater frequency than other polymorphic markers and are spaced with a greater uniformity throughout a genome than other reported forms of polymorphism. The greater frequency and uniformity of SNPs means that there is greater probability that such a polymorphism will be found near or in a genetic locus of interest than would be the case for other polymorphisms. SNPs are located in protein-coding regions and noncoding regions of a genome. Some of these SNPs may result in defective or variant protein expression (*e.g.*, as a result of mutations or defective splicing). Analysis (genotyping) of characterized SNPs can require only a plus/minus assay rather than a lengthy measurement, permitting easier automation.

SNPs can be characterized using any of a variety of methods. Such methods include the direct or indirect sequencing of the site, the use of restriction enzymes (Botstein *et al., Am. J. Hum. Genet. 32*:314-331 (1980); Konieczny and Ausubel, *Plant J. 4*:403-410 (1993)), enzymatic and chemical mismatch assays (Myers *et al., Nature 313*:495-498 (1985)), allele-specific PCR (Newton *et al., Nucl. Acids Res. 17*:2503-2516 (1989); Wu *et al., Proc. Natl. Acad. Sci. USA 86*:2757-2760 (1989)), ligase chain reaction (Barany, *Proc. Natl. Acad. Sci. USA 88*:189-193 (1991)), single-strand conformation polymorphism analysis (Labrune *et al., Am. J. Hum. Genet. 48*:1115-1120 (1991)), single base primer extension (Kuppuswamy *et al., Proc. Natl. Acad. Sci. USA 88*:1143-1147 (1991)), Goelet US 6,004,744; Goelet 5,888,819), solid-phase ELISA-based oligonucleotide ligation assays (Nikiforov *et al., Nucl. Acids Res. 22*:4167-4175 (1994), dideoxy fingerprinting (Sarkar *et al., Genomics 13*:441-443 (1992)), oligonucleotide fluorescence-quenching assays (Livak *et al., PCR Methods Appl. 4*:357-362 (1995a)), 5'-nuclease allele-specific hybridization TaqMan™ assay (Livak *et al., Nature Genet. 9*:341-342 (1995)), template-directed dye-terminator incorporation (TDI) assay (Chen and Kwok, *Nucl. Acids Res. 25*:347-353

(1997)), allele-specific molecular beacon assay (Tyagi *et al.*, *Nature Biotech.* *16*:49-53 (1998)), PinPoint assay (Haff and Smirnov, *Genome Res.* *7*:378-388 (1997)), dCAPS analysis (Neff *et al.*, *Plant J.* *14*:387-392 (1998)), pyrosequencing (Ronaghi *et al*, *Analytical Biochemistry 267*:65-71 (1999); Ronaghi *et al* PCT application WO 98/13523; Nyren *et al* PCT application WO 98/28440; www.pyrosequencing.com), using mass spectrometry, *e.g.* the Masscode ™ system (Howbert *et al* PCT application, WO 99/05319; Howbert *et al* PCT application WO 97/27331; www.rapigene.com; Becker *et al* PCT application WO 98/26095; Becker *et al* PCT application; WO 98/12355; Becker *et al* PCT application WO 97/33000; Monforte *et al* US 5,965,363), invasive cleavage of oligonucleotide probes (Lyamichev *et al Nature Biotechnology 17*:292-296; www.twt.com), and using high density oligonucleotide arrays (Hacia *et al Nature Genetics 22*:164-167; www.affymetrix.com).

Polymorphisms may also be detected using allele-specific oligonucleotides (ASO), which, can be for example, used in combination with hybridization based technology including Southern, Northern, and dot blot hybridizations, reverse dot blot hybridizations and hybridizations performed on microarray and related technology.

The stringency of hybridization for polymorphism detection is highly dependent upon a variety of factors, including length of the allele-specific oligonucleotide, sequence composition, degree of complementarity (*i.e.* presence or absence of base mismatches), concentration of salts and other factors such as formamide, and temperature. These factors are important both during the hybridization itself and during subsequent washes performed to remove target polynucleotide that is not specifically hybridized. In practice, the conditions of the final, most stringent wash are most critical. In addition, the amount of target polynucleotide that is able to hybridize to the allele-specific oligonucleotide is also governed by such factors as the concentration of both the ASO and the target polynucleotide, the presence and concentration of factors that act to "tie up" water molecules, so as to effectively concentrate the reagents (*e.g.*, PEG, dextran, dextran sulfate, *etc.*), whether the nucleic acids are immobilized or in solution, and the duration of hybridization and washing steps.

Hybridizations are preferably performed below the melting temperature (T_m) of the ASO. The closer the hybridization and/or washing step is to the T_m, the higher the stringency. T_m for an oligonucleotide may be approximated, for example, according to the following formula: $T_m = 81.5 + 16.6 \times (\log10[Na+]) + 0.41 \times (\%G+C) - 675/n$; where

[Na+] is the molar salt concentration of Na+ or any other suitable cation and n = number of bases in the oligonucleotide. Other formulas for approximating T_m are available and are known to those of ordinary skill in the art.

Stringency is preferably adjusted so as to allow a given ASO to differentially hybridize to a target polynucleotide of the correct allele and a target polynucleotide of the incorrect allele. Preferably, there will be at least a two-fold differential between the signal produced by the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele (*e.g.*, an ASO specific for a mutant allele cross-hybridizing to a wild-type allele). In more preferred embodiments of the present invention, there is at least a five-fold signal differential. In highly preferred embodiments of the present invention, there is at least an order of magnitude signal differential between the ASO hybridizing to a target polynucleotide of the correct allele and the level of the signal produced by the ASO cross-hybridizing to a target polynucleotide of the incorrect allele.

While certain methods for detecting polymorphisms are described herein, other detection methodologies may be utilized. For example, additional methodologies are known and set forth, in Birren *et al.*, *Genome Analysis,* 4:135-186, *A Laboratory Manual. Mapping Genomes*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999); Maliga *et al.*, *Methods in Plant Molecular Biology. A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1995); Paterson, *Biotechnology Intelligence Unit: Genome Mapping in Plants*, R.G. Landes Co., Georgetown, TX, and Academic Press, San Diego, CA (1996); *The Corn Handbook*, Freeling and Walbot, eds., Springer-Verlag, New York, NY (1994); *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Elles, ed., Humana Press, Totowa, NJ (1996); Clark, ed., *Plant Molecular Biology: A Laboratory Manual*, Clark, ed., Springer-Verlag, Berlin, Germany (1997).

Factors for marker-assisted selection in a plant breeding program are: (1) the marker(s) should co-segregate or be closely linked with the desired trait; (2) an efficient means of screening large populations for the molecular marker(s) should be available; and (3) the screening technique should have high reproducibility across laboratories and preferably be economical to use and be user-friendly.

The genetic linkage of marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics 121*:185-199 (1989) and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics 121*:185-199 (1989) and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL*, Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A $\log_{10}$ of an odds ratio (LOD) is then calculated as: LOD = $\log_{10}$ (MLE for the presence of a QTL/MLE given no linked QTL).

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein, *Genetics 121*:185-199 (1989) and further described by Arús and Moreno-González, *Plant Breeding*, Hayward *et al.*, (eds.) Chapman & Hall, London, pp. 314-331 (1993).

In a preferred embodiment of the present invention the nucleic acid marker exhibits a LOD score of greater than 2.0, more preferably 2.5, even more preferably greater than 3.0 or 4.0 with the trait or phenotype of interest. In a preferred embodiment, the trait of interest is altered tocopherol levels or compositions or altered tocotrienol levels or compositions.

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use of non-parametric methods (Kruglyak and Lander, *Genetics 139:*1421-1428 (1995)). Multiple regression methods or models can also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breeding*, van Oijen and Jansen (eds.), Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.

116-124 (1994); Weber and Wricke, *Advances in Plant Breeding,* Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics 136*:1447-1455 (1994), and Zeng, *Genetics 136*:1457-1468 (1994). Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding,* van Oijen and Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics 136*:1457-1468 (1994)). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al., Theo. Appl. Genet. 91*:33-37 (1995)).

It is understood that one or more of the nucleic acid molecules of the invention may be used as molecular markers. It is also understood that one or more of the protein molecules of the invention may be used as molecular markers.

In a preferred embodiment, the polymorphism is present and screened for in a mapping population, *e.g.* a collection of plants capable of being used with markers such as polymorphic markers to map genetic position of traits. The choice of appropriate mapping population often depends on the type of marker systems employed (Tanksley *et al., J.P. Gustafson and R. Appels* (eds.). Plenum Press, New York, pp. 157-173 (1988)). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large number of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An F_2 population is the first generation of selfing (self-pollinating) after the hybrid seed is produced. Usually a single F_1 plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) pattern. Maximum genetic information is obtained from a completely classified F_2 population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co., (1938)). In the case of dominant markers, progeny tests (*e.g.*, F_3, BCF_2) are required to identify the heterozygotes, in order to classify the population. However, this procedure is often prohibitive because of the cost

and time involved in progeny testing. Progeny testing of F_2 individuals is often used in map construction where phenotypes do not consistently reflect genotype (*e.g.* disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations *e.g.* F_3 or BCF_2) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F_2, F_3), where linkage groups have not been completely disassociated by recombination events (*i.e.*, maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually >F_5, developed from continuously selfing F_2 lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about <10% recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter. *Proc. Natl. Acad. Sci. (U.S.A.) 89*:1477-1481 (1992)). However, as the distance between markers becomes larger (*i.e.*, loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al., Proc. Natl. Acad. Sci. (U.S.A.) 89*:1477-1481 (1992)). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from F_2 populations because one, rather than two, recombinant gamete is sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (*i.e.* about 0.15% recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) (created by many backcrosses to produce a collection of individuals that is nearly identical in genetic composition except for the trait or genomic region under interrogation) can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci is expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*:9828-9832 (1991)). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular disease) or genomic region but arbitrary at unlinked regions (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

In an aspect of the present invention, one or more of the nucleic molecules of the present invention are used to determine the level (*i.e.*, the concentration of mRNA in a sample, *etc.*) in a plant (preferably canola, corn, *Brassica campestris, Brassica napus,* oilseed rape, rapeseed, soybean, crambe, mustard, castor bean, peanut, sesame, cottonseed, linseed, safflower, oil palm, flax or sunflower) or pattern (*i.e.*, the kinetics of expression, rate of decomposition, stability profile, *etc.*) of the expression of a protein encoded in part or whole by one or more of the nucleic acid molecule of the present invention (collectively, the "Expression Response" of a cell or tissue).

As used herein, the Expression Response manifested by a cell or tissue is said to be "altered" if it differs from the Expression Response of cells or tissues of plants not exhibiting the phenotype. To determine whether a Expression Response is altered, the Expression Response manifested by the cell or tissue of the plant exhibiting the phenotype is compared with that of a similar cell or tissue sample of a plant not exhibiting the phenotype. As will be appreciated, it is not necessary to re-determine the Expression Response of the cell or tissue sample of plants not exhibiting the phenotype each time such a comparison is made; rather, the Expression Response of a particular plant may be compared with previously obtained values of normal plants. As used herein, the phenotype of the organism is any of one or more characteristics of an organism (*e.g.* disease resistance, pest tolerance, environmental tolerance such as tolerance to abiotic stress, male sterility, quality improvement or yield *etc.*). A change in genotype or phenotype may be transient or permanent. Also as used herein, a tissue sample is any sample that comprises

more than one cell. In a preferred aspect, a tissue sample comprises cells that share a common characteristic (e.g. Derived from root, seed, flower, leaf, stem or pollen etc.).

In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid molecules of the present invention are utilized to detect the presence or quantity of the mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

A number of methods can be used to compare the expression response between two or more samples of cells or tissue. These methods include hybridization assays, such as northerns, RNAse protection assays, and *in situ* hybridization. Alternatively, the methods include PCR-type assays. In a preferred method, the expression response is compared by hybridizing nucleic acids from the two or more samples to an array of nucleic acids. The array contains a plurality of suspected sequences known or suspected of being present in the cells or tissue of the samples.

An advantage of *in situ* hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer *et al., Dev. Biol. 101*:477-484 (1984); Angerer *et al., Dev. Biol. 112*:157-166 (1985); Dixon *et al., EMBO J. 10*:1317-1324 (1991)). *In situ* hybridization may be used to measure the steady-state level of RNA accumulation (Hardin *et al., J. Mol. Biol. 202*:417-431 (1989)). A number of protocols have been devised for *in situ* hybridization, each with tissue preparation, hybridization and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep. 5*:242-250 (1987); Cox and Goldberg, In: *Plant Molecular Biology: A Practical Approach,* Shaw (ed.), pp. 1-35, IRL Press, Oxford (1988); Raikhel *et al., In situ RNA hybridization in plant tissues*, In: *Plant Molecular Biology Manual*, vol. B9:1-32, Kluwer Academic Publisher, Dordrecht, Belgium (1989)).

In situ hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization,* Oxford University Press, Oxford (1992); Langdale, *In Situ Hybridization* In: *The Corn Handbook,* Freeling and Walbot (eds.), pp. 165-179, Springer-Verlag, New York (1994)). It is understood that one or more of the molecules of the invention, preferably one or more of the nucleic acid molecules or fragments thereof of the invention or one or more of the antibodies of the invention may be utilized to detect the level or pattern of a protein or mRNA thereof by *in situ* hybridization.

Fluorescent *in situ* hybridization allows the localization of a particular DNA sequence along a chromosome, which is useful, among other uses, for gene mapping, following chromosomes in hybrid lines, or detecting chromosomes with translocations, transversions or deletions. *In situ* hybridization has been used to identify chromosomes in several plant species (Griffor *et al., Plant Mol. Biol. 17*:101-109 (1991); Gustafson *et al., Proc. Natl. Acad. Sci. (U.S.A.) 87*:1899-1902 (1990); Mukai and Gill, *Genome 34*:448-452 (1991); Schwarzacher and Heslop-Harrison, *Genome 34*:317-323 (1991); Wang *et al., Jpn. J. Genet. 66*:313-316 (1991); Parra and Windle, *Nature Genetics 5*:17-21 (1993)). It is understood that the nucleic acid molecules of the invention may be used as probes or markers to localize sequences along a chromosome.

Another method to localize the expression of a molecule is tissue printing. Tissue printing provides a way to screen, at the same time on the same membrane many tissue sections from different plants or different developmental stages (Yomo and Taylor, *Planta 112*:35-43 (1973); Harris and Chrispeels, *Plant Physiol.* 56:292-299 (1975); Cassab and Varner, *J. Cell. Biol. 105*:2581-2588 (1987); Spruce *et al., Phytochemistry 26*:2901-2903 (1987); Barres *et al., Neuron 5*:527-544 (1990); Reid and Pont-Lezica, *Tissue Printing: Tools for the Study of Anatomy, Histochemistry and Gene Expression,* Academic Press, New York, New York (1992); Reid *et al., Plant Physiol. 93*:160-165 (1990); Ye *et al., Plant J. 1*:175-183 (1991)).

One skilled in the art can refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include *Current Protocols in Molecular Biology* Ausubel, *et al.,* eds., John Wiley & Sons, N.Y. (1989), and supplements through September (1998), *Molecular Cloning, A Laboratory Manual,* Sambrook *et al,* 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), *Genome Analysis: A Laboratory Manual 1: Analyzing DNA,* Birren *et al.,* Cold Spring Harbor Press, Cold Spring Harbor, New York (1997); *Genome Analysis: A Laboratory*

Manual 2: Detecting Genes, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1998); *Genome Analysis: A Laboratory Manual 3: Cloning Systems*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Genome Analysis: A Laboratory Manual 4: Mapping Genomes*, Birren *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1999); *Plant Molecular Biology: A Laboratory Manual*, Clark, Springer-Verlag, Berlin, (1997), *Methods in Plant Molecular Biology*, Maliga *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1995). These texts can, of course, also be referred to in making or using an aspect of the invention. It is understood that any of the agents of the invention can be substantially purified and/or be biologically active and/or recombinant.

Having now generally described the invention, the same will be more readily understood through reference to the following examples that are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1.

Identification and characterization of mutant hdt2 *Arabidopsis thaliana*, ecotype Landsberg plants.

Mutagenized (M_2) seeds of *Arabidopsis thaliana*, ecotype Landsberg are obtained both by purchase from Lehle Seeds (Round Rock, Texas, U.S.A.) and by standard EMS mutagenesis methodology. The M_2 plants are grown from the M_2 seeds in greenhouse conditions with one plant per 2.5 inch pot. The resulting M_3 seeds are collected from individual M_2 plants and analyzed for tocopherol levels.

Seeds from approximately 10,000 M_3 lines of *Arabidopsis thaliana*, ecotype Landsberg or Col-O are analyzed for individual tocopherol levels using the following procedure. Five milligrams of seeds from individual plants are ground to a fine powder using a 1/8" steel ball bearing and vigorous shaking. 200 Microliters of 99.5% ethanol/0.5% pyrogallol is added, mixed for 30 seconds and allowed to incubate at 4°C for 1h. 50 Microgram/ml of tocol (Matreya, Inc., Pleasant Gap, PA) is added to each sample as an injection standard. To remove debris following centrifugation, the supernatant is filtered (PVDF 0.45 µm, Whatman). The filtrate is then analyzed for tocopherol content using high performance liquid chromatography (HPLC) using an isocratic gradient of 90% hexane/10% methyl-t-butyl ether with a Zorbax silica column (4.6 x 250 mm, Agilent Technologies, Atlanta, GA) and fluorescence detection (model 2790 HPLC with model 474

detector; Waters Corporation, Bedford, MA) (excitation at 290 nm, emit at 336 nm, 30 nm bandpass and slits). Levels of α, β, γ, and δ-tocopherol are measured in addition to tocol, the injection standard. Individual plant lines that have δ-tocopherol levels higher than wild type are reanalyzed in the next generation (M4), to confirm their inheritability. Five *Arabidopsis* high δ-tocopherol (hdt) mutants possessing increased levels of δ-tocopherols, as compared to wild type, are isolated.

Table 1 below shows the percentage, on a dry weight basis, of δ-tocopherol levels and the relative increases over the appropriate wild type parental ecotype for each of the six mutants. The results show that the six mutants have significant increases in δ-tocopherol levels when compared to the corresponding wild type control. The magnitude of the increases ranged from 2-25 fold.

Table 1

Mutant	WT ecotype	Delta Composition	Increase over WT
hdt2	Ler	48%	25 fold
hdt6	Col-0	45%	20 fold
hdt9	Col-0	6%	2 fold
hdt10	Ler	25%	7 fold
hdt16	Col-0	50%	17 fold

Example 2.

Identification and sequencing of the mutant hdt2 gene in the *Arabidopsis thaliana*, Landsberg *erecta* (Ler) high δ-tocopherol mutants.

Using map-based cloning techniques (see, for example, USSN 09/803,736, Plant Polymorphic Markers and Uses Thereof, filed March 12, 2001) the mutant *hdt2* gene is mapped to chromosome 3 telomeric marker T12C14_1563 at 85 cM. This region contains approximately 60 predicted genes. Our analysis of the genes in this region revealed that one of the genes, MAA21_40, possesses homology to known ubiquinone methyltransferases. Based on this homology and the prediction that MAA21_40 is targeted to the chloroplast, this gene is determined to be likely to contain the mutation responsible for the high δ-tocopherol phenotype in hdt2 mutants. The sequences of the MAA21_40 gene locus in the wild types and hdt2 mutants are PCR amplified, and determined by

standard sequencing methodology. The gene locus, in each case, is amplified using the sequencing primers as described below:

Primer Pair Name MAA21_40_1

Forward Primer TGTAAAACGACGGCCAGTTGCTGAAAGTTGAAAAGAGCAA (SEQ ID NO: 55)

Reverse Primer CAGGAAACAGCTATGACCCAATTTGATCAATGTTCCACGA (SEQ ID NO: 56)

Primer Pair Name MAA21_40_2

Forward Primer TGTAAAACGACGGCCAGTAGCTATGCGGATTGATGGTC (SEQ ID NO: 57)

Reverse Primer CAGGAAACAGCTATGACCTCCTCCTGGGAACTCTAGCA (SEQ ID NO: 58)

Primer Pair Name MAA21_40_3

Forward Primer TGTAAAACGACGGCCAGTTGCTGACTTGCGAGTTTTTG (SEQ ID NO: 59)

Reverse Primer CAGGAAACAGCTATGACCCCTGTCAACAACCCCTTCTC (SEQ ID NO: 60)

Primer Pair Name MAA21_40_4

Forward Primer TGTAAAACGACGGCCAGTCCACAAGAGGGGTTTACAATG (SEQ ID NO: 61)

Reverse Primer CAGGAAACAGCTATGACCACCCAACCTTCTGGCTCTCT (SEQ ID NO: 62)

Primer Pair Name MAA21_40_5

Forward Primer TGTAAAACGACGGCCAGTGGTCTTTGGGAACGATCTGA (SEQ ID NO: 63)

Reverse Primer CAGGAAACAGCTATGACCAGGGAAGCGTACAGGGTTCT (SEQ ID NO: 64)

Primer Pair Name MAA21_40_6

Forward Primer TGTAAAACGACGGCCAGTCCTCTTGAGCTGAACGTCCT (SEQ ID NO: 65)

Reverse Primer CAGGAAACAGCTATGACCGGCGGAACTGGTTTCACTAC (SEQ ID NO: 66)

Primer Pair Name MAA21_40_7

Forward Primer TGTAAAACGACGGCCAGTTGTCAGCATAATCGGTTGGA (SEQ ID NO: 67)

Reverse Primer CAGGAAACAGCTATGACCTCCCCAAAGGTTTAGGTTCC (SEQ ID NO: 68)

Primer Pair Name MAA21_40_8

Forward Primer TGTAAAACGACGGCCAGTAAGCCTCCTTCTTGTGCTGA (SEQ ID NO: 69)

Reverse Primer CAGGAAACAGCTATGACCCGACTTTTCCCTTCCATTTG (SEQ ID NO: 70)

Primer Pair Name MAA21_40_9

Forward Primer TGTAAAACGACGGCCAGTTGGAGGTTCGGGTAACTGAG (SEQ ID NO: 71)

Reverse Primer CAGGAAACAGCTATGACCCATCCTCTCGCTAGCAGGTC (SEQ ID NO: 72)

Primer Pair Name MAA21_40_10

Forward Primer TGTAAAACGACGGCCAGTGGAACCAGGGGAACCTAAAC (SEQ ID NO: 73)

Reverse Primer CAGGAAACAGCTATGACCGCCGTGAGAAACAGACTCCT (SEQ ID NO: 74)

Primer Pair Name MAA21_40_11

Forward Primer TGTAAAACGACGGCCAGTCAAATGGAAGGGAAAAGTCG (SEQ ID NO: 75)

Reverse Primer CAGGAAACAGCTATGACCGATCCAAAGAGAACCCAGCA (SEQ ID NO: 76)

The following Polymerase Chain Reaction (PCR) mixture is prepared for each primer pair:

PCR mixture:

5 μl 10X Taq Buffer

5 μl 25mM $MgCl_2$

4 μl 10mM dNTPs

2 μl Template DNA

0.5 μl Taq Gold

5 μl F/R Sequencing Primers

28.5 μl dH_2O

The PCR amplification is carried out using the following Thermocycler program:

1. 94 °C for 10 minutes
2. 94 °C for 15 seconds
3. 56 °C for 15 seconds
4. 72 °C for 1 minute, 30 seconds
5. Repeat Steps 2 through 4 an additional 44 times
6. 72 °C for 10 minutes
7. Hold at 4 °C

The resulting PCR products are sequenced using standard sequencing methodologies.

The wild type Col-0 genomic sequence for the MAA21_40 locus is set forth in SEQ ID NO: 1. The wild type Ler genomic sequence for the MAA21_40 locus is set forth in SEQ ID NO: 2. The wild type coding DNA and peptide sequence for Columbia and Landsberg ecotypes are described in SEQ ID NOs: 15 and 16, respectively.

Once the sequences of the MAA21_40 gene from the hdt2 mutant are determined, they are compared to the sequence of the wild type gene. The high δ-tocopherol mutant identified as hdt2 is determined to have a MAA21_40 gene with the nucleic acid sequence

set forth in SEQ ID NO: 3. This sequence has a glutamate to lysine substitution at amino acid position 292, relative to the ATG of the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 17.

Another high δ-tocopherol mutant, identified as hdt6, is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 4. This sequence has a glutamate to a lysine substitution at amino acid 72, relative to the wild type *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 18.

Another high δ-tocopherol mutant, identified as hdt9 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 5. This sequence has a proline to a serine substitution at amino acid 13, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 19.

Another high δ-tocopherol mutant, identified as hdt10 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 6 which encodes MAA21_40 with a aspartate to a asparagine substitution at amino acid 116, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 20.

Another high δ-tocopherol mutant hdt16 is determined to have a MAA21_40 gene with the nucleic acid sequence set forth in SEQ ID NO: 7 which encodes MAA21_40 with a threonine to an isoleucine substitution at amino acid 94, relative to the *Arabidopsis* MAA21_40, as shown in the amino acid sequence of SEQ ID NO: 21.

Table 2 summarizes the mutations described above.

Table 2

Mutant	Nucleotide Mutation	Amino Acid Change
hdt2	G1041A	E292K
hdt6	G214A	E72K
hdt9	C37T	P13S
hdt10	G346A	D116N
hdt16	C281T	T94I

Example 3.

Identification of genes from various sources demonstrating homology to the tMT2 gene from *Arabidopsis thaliana*.

The protein sequence of tMT2 from *Arabidopsis thaliana* (NCBI General Identifier Number gi7573324) is used to search databases for plant sequences with homology to tMT2 using TBLASTN (Altschul *et al.*, *Nucleic Acids Res.* *25*:3389-3402 (1997); see also www.ncbi.nlm.nih.gov/BLAST/). Nucleic acid sequences SEQ ID NO: 8 through 15 are found to have high homology with the *Arabidopsis* sequence..

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>CPR19219 Brassica napus tMT2 homolog 1 - LIB4153-013-R1-K1-B7
ATGGCTTCTCTCATGCTCAACGGGGCCATCACCTTCCCCAAGGGATTAGGCTTCCCCGCTTCCAATCTACACG
CCAGACCAAGTCCTCCGCTGAGTCTCGTCTCAAACACAGCCACGCGGAGACTCTCCGTGGCGACAAGATGCAG
CAGCAGCAGCAGCGTGTCGGCGTCAAGGCCATCTGCGCAGCCTAGGTTCATCCAGCACAAGAAAGAGGCCTAC
TGGTTCTACAGGTTCCTGTCCATCGTGTACGACCACATCATCAATCCCGGCCACTGGACGGAGGATATGAGGG
ACGACGCTCTCGAGCCTGCGGATCTGAGCCATCCGGACATGCGAGTTGTCGACGTCGGAGGCGGAACGGGTTT
CACCACGCTGGGAATCGTCAAGACGGTGAAGGCTAAGAACGTGACGATTCTGGACCAGTCGCCGCATCAGCTG
GCAAAGGCGAAGCAGAAGGAGCCGTTGAAGGAGTGCAAGATCGTTGAAGGAGATGCGGAGGATCTCCCTTTTC
CTACTGATTATGCTGACAGATACGTCTCTGCTGGAAGCATTGAGTACTGGCCCGACCCGCAGAGGGGGATAAG
GGAAGCGTACAGAGTTCTCAAGATCGGTGGGAAAGCATGTCTCATTGGCCCTGTCCACCCGACGTTTTGGCTT
TCTCGTTTCTTTGCAGATGTGTGGATGCTTTTCCCCAAGGAGGAGGAGTACATTGAGTGGTTCAAGAATGCTG
GTTTCAAGGACGTTCAGCTTAAGAGGATTGGCCCCAAGTGGTACCGTGGTGTTCGCAGGCACGGACTTATCAT
GGGATGCTCTGTTACTGGTGTCAAACCTGCCTCTGGAGACTCTCCTCTCCAGCTTGGACCAAAGGAAGAGGAC
GTGGAGAAGCCTGTAAACAATCCTTTCTCCTTCTTGGGACGCTTCCTCTTGGGAACCTTAGCGGCTGCCTGGT
TTGTGTTAATCCCAATCTACATGTGGATCAAGGATCAGATCGTTCCCAAAGACCAACCCATCTGA (SEQ ID
NO: 13)

>Protein sequence Brassica napus tMT2 homolog 1 - LIB4153-013-R1-K1-B7
MASLMLNGAITFPKGLGFPASNLHARPSPPLSLVSNTATRRLSVATRCSSSSSVSASRPSAQPRFIQHKKEAY
WFYRFLSIVYDHIINPGHWTEDMRDDALEPADLSHPDMRVVDVGGGTGFTTLGIVKTVKAKNVTILDQSPHQL
AKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQRGIREAYRVLKIGGKACLIGPVHPTFWL
SRFFADVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPASGDSPLQLGPKEED
VEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI (SEQ ID NO: 27)

>CPR19220 Brassica napus tMT2 homolog 2 - LIB80-011-Q1-E1-E9
ATGGCTTCTCTCATGCTCAACGGGGCCATCACCTTCCCCAAGGGATTAGGCTTCCCCGCTTCCAATCTACACG
CCAGACCAAGTCCTCCGCTGAGTCTCGTCTCAAACACAGCCACGCGGAGACTCTCCGTGGCGACAAGATGCAG
CAGCAGCAGCAGCGTGTCGGCGTCAAGGCCATCTGCGCAGCCTAGGTTCATCCAGCACAAGAAAGAGGCCTAC
TGGTTCTACAGGTTCCTGTCCATCGTGTACGACCACATCATCAATCCCGGCCACTGGACGGAGGATATGAGGG
ACGACGCTCTCGAGCCTGCGGATCTGAGCCATCCGGACATGCGAGTTGTCGACGTCGGAGGCGGAACGGGTTT
CACCACGCTGGGAATCGTCAAGACGGTGAAGGCTAAGAACGTGACGATTCTGGACCAGTCGCCGCATCAGCTG
GCAAAGGCGAAGCAGAAGGAGCCGTTGAAGGAGTGCAAGATCGTGGAAGGAGATGCGGAGGATCTCCCTTTTC
CTACTGATTATGCTGACAGATACGTCTCTGCTGGAAGCATTGAGTACTGGCCCGACCCGCAGAGGGGTATAAG
GGAAGCGTACAGAGTTCTCAAGATCGGTGGGAAAGCATGTCTCATTGGCCCTGTCCACCCGACGTTTTGGCTT
TCACGCTTCTTTGCAGATGTGTGGATGCTTTTCCCCAAGGAGGAGGAGTACATTGAGTGGTTCAAGAATGCTG
GTTTCAAGGACGTTCAGCTTAAGAGGATTGGCCCCAAGTGGTACCGTGGTGTTCGCAGGCACGGACTTATCAT
GGGATGCTCTGTTACTGGTGTCAAACCTGCCTCTGGAGACTCTCCTCTCCAGCTTGGACCAAAGGAAGAGGAC
GTGGAGAAGCCTGTAAACAATCCTTTCTCCTTCTTGGGACGCTTCCTCTTGGGTACCCTAGCGGCTGCCTGGT
TTGTGTTAATCCCAATCTACATGTGGATCAAGGATCAGATCGTTCCCAAAGACCAACCCATCTGA (SEQ ID
NO: 14)

> CPR 193223 Oryza sativa tMT2- LIB4371-041-R1-K1-F7
ATGGCGATGGCCTCCTCCGCCTACGCCCCAGCGGGCGGCGTTGGCACCCACTCCGCGCCGGGCAGGATCAGGC
CGCCGCGCGGCCTCGGCTTCTCCACCACCACCACCAAGTCGAGGCCCCTCGTGCTCACCAGGCGTGGGGGAGG
CGGCGGCAACATCTCCGTGGCTCGGCTGAGGTGCGCGGCGTCGTCGTCGTCGGCGGCGGCGAGGCCGATGTCG
CAGCCGCGGTTCATCCAGCACAAGAAGGAGGCGTTCTGGTTCTACCGCTTCCTCTCCATCGTCTACGACCACG
TCATCAACCCGGGCCACTGGACGGAGGACATGCGGGACGACGCCCTCGAGCCCGCCGACCTCTACAGCCGCAA
GCTCAGGGTCGTCGACGTCGGCGGCGGGACGGGGTTCACCACGCTCGGGATCGTCAAGCGCGTCGACCCGGAG
AACGTCACGCTGCTCGACCAGTCCCCGCACCAGCTCGAGAAGGCCCGGGAGAAGGAGGCCCTCAAGGGCGTCA
CCATCATGGAGGGCGACGCCGAGGACCTCCCCTTCCCCACCGACACCTTCGACCGCTACGTCTCCGCCGGCAG
CATCGAGTATTGGCCCGATCCGCAGCGAGGAATCAAGGAAGCTTACAGGGTTTTGAGGCTTGGTGGAGTGGCT
```

TGCATGATTGGCCCCGTGCACCCAACCTTCTGGCTGTCTCGCTTTTTCGCTGACATGTGGATGCTCTTCCCGA
AGGAAGAGGAGTATATTGAGTGGTTCAAAAAGGCAGGGTTCAAGGATGTCAAGCTCAAAAGGATTGGACCAAA
ATGGTACCGTGGTGTCCGAAGGCATGGCCTGATTATGGGATGCTCTGTGACGGGCGTCAAAAGAGAACATGGA
GACTCCCCTTTGCAGCTTGGTCCAAAGGTTGAGGATGTCAGCAAACCTGTGAATCCTATCACCTTCCTCTTCC
GCTTCCTCATGGGAACAATATGTGCTGCATACTATGTTCTGGTGCCTATCTACATGTGGATAAAGGACCAGAT
TGTGCCCAAAGGCATGCCGATCTAA(SEQ ID NO: 12)

> Protein translation *Oryza sativa* tMT2 - LIB4371-041-R1-K1-F7
MAMASSAYAPAGGVGTHSAPGRIRPPRGLGFSTTTTKSRPLVLTRRGGGGGNISVARLRCAASSSSAAARPMS
QPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLYSRKLRVVDVGGGTGFTTLGIVKRVDPE
NVTLLDQSPHQLEKAREKEALKGVTIMEGDAEDLPFPTDTFDRYVSAGSIEYWPDPQRGIKEAYRVLRLGGVA
CMIGPVHPTFWLSRFFADMWMLFPKEEEYIEWFKKAGFKDVKLKRIGPKWYRGVRRHGLIMGCSVTGVKREHG
DSPLQLGPKVEDVSKPVNPITFLFRFLMGTICAAYYVLVPIYMWIKDQIVPKGMPI (SEQ ID NO: 26)

> CPR193225 and 193226 *Zea mays* tMT2- LIB3587-273-Q1-K6-C5/ LIB3600-046-Q1-K6-G1
ATGGCGATGGCCTCCACCTACGCGCCGGGCGGAGGCGCGCGGGCGCTCGCGCAGGGTAGATGCAGGGTCCGCG
GTCCCGCGGGGCTGGGCTTCCTCGGCCCCTCCAAGGCCGCCGGCCTCCCCCGCCCCCTCGCCCTCGCCCTCGC
CAGGCGGATGAGCAGCCCCGTCGCGGTGGGCGCCAGGCTGCGATGCGCGGCGTCGTCGTCCCCCGCGGCGGCG
CGGCCCGCCACGGCGCCGCGCTTCATCCAGCACAAGAAGGAGGCCTTCTGGTTCTACCGCTTCCTCTCCATCG
TGTACGACCACGTCATCAATCCGGGCCACTGGACCGAGGACATGCGCGACGACGCGCTGGAACCTGCCGACCT
CTTCAGCCGCCACCTCACGGTCGTCGACGTCGGCGGCGGCACGGGGTTCACCACGCTCGGCATCGTCAAGCAC
GTCAACCCGGAGAACGTCACGCTGCTCGACCAGTCCCCGCACCAGCTCGACAAGGCCCGGCAGAAGGAGGCCC
TCAAGGGGGTCACCATCATGGAGGGCGACGCCGAGGACCTCCCGTTCCCCACCGACTCCTTCGACCGATACAT
CTCCGCCGGCAGCATCGAGTACTGGCCAGACCCACAGCGGGGGATCAAGGAAGCCTACAGGGTCCTGAGATTT
GGTGGGCTAGCTTGTGTGATCGGCCCGGTCTACCCGACCTTCTGGCTGTCCCGCTTCTTCGCCGACATGTGGA
TGCTCTTCCCCAAGGAGGAAGAGTACATCGAGTGGTTCAAGAAGGCTGGGTTTAGGGATGTCAAGCTGAAGAG
GATTGGACCGAAGTGGTACCGCGGTGTCCGAAGGCATGGCCTCATCATGGGCTGCTCCGTCACAGGCGTCAAG
AGAGAGCGCGGTGACTCTCCCTTGGAGCTTGGTCCCAAGGCGGAGGATGTCAGCAAGCCAGTGAATCCGATCA
CCTTCCTCTTCCGCTTCCTCGTAGGAACGATATGTGCTGCCTACTATGTTCTGGTGCCTATTTACATGTGGAT
AAAGGACCAGATCGTGCCAAAAGGCATGCCAATCTGA (SEQ ID NO: 8)

> Protein translation *Zea mays* tMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-Q1-K6-G1
MAMASTYAPGGGARALAQGRCRVRGPAGLGFLGPSKAAGLPRPLALALARRMSSPVAVGARLRCAASSSPAAA
RPATAPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLFSRHLTVVDVGGGTGFTTLGIVKH
VNPENVTLLDQSPHQLDKARQKEALKGVTIMEGDAEDLPFPTDSFDRYISAGSIEYWPDPQRGIKEAYRVLRF
GGLACVIGPVYPTFWLSRFFADMWMLFPKEEEYIEWFKKAGFRDVKLKRIGPKWYRGVRRHGLIMGCSVTGVK
RERGDSPLELGPKAEDVSKPVNPITFLFRFLVGTICAAYYVLVPIYMWIKDQIVPKGMPI(SEQ ID NO: 22)

>CPR193234 *Glycine max* tMT2 - LIB3049-032-Q1-E1-G8
ATGGGTTCAGTAATGCTCAGTGGAACTGAAAAGCTCACTCTCAGAACCCTAACCGGGAACGGCTTAGGTTTCA
CTGGTTCGGATTTGCACGGTAAGAACTTCCCAAGAGTGAGTTTCGCTGCTACCACTAGTGCTAAAGTTCCCAA
CTTTAGAAGCATAGTAGTACCCAAGTGTAGTGTCTCGGCTTCCAGGCCAAGCTCGCAGCCAAGGTTCATTCAG
CACAAAAAAGAGGCCTTTTGGTTCTATAGGTTTCTCTCAATTGTGTATGACCATGTCATTAACCCTGGCCATT
GGACCGAGGACATGAGGGATGATGCCCTTGAACCCGCTGATCTCAATGACAGGAACATGATTGTGGTGGATGT
TGGTGGCGGCACGGGTTTCACCACTCTTGGTATTGTCAAGCACGTGGATGCCAAGAATGTCACCATTCTTGAC
CAGTCACCCCACCAGCTCGCCAAGGCCAAGCAGAAGGAGCCACTCAAGGAATGCAAAATAATCGAAGGGGATG
CCGAGGATCTCCCCTTTCGAACTGATTATGCCGATAGATATGTATCCGCAGGAAGTATTGAGTACTGGCCGGA
TCCACAGCGTGGCATCAAGGAGGCATACAGGGTTTTGAAACTTGGAGGCAAAGCGTGTCTAATTGGTCCGGTC
TACCCAACATTTTGGTTGTCACGTTTCTTTGCAGATGTTTGGATGCTTTTCCCCAAGGAGGAAGAGTATATTG
AGTGGTTTCAGAAGGCAGGGTTTAAGGACGTCCAACTAAAAAGGATTGGCCCAAAATGGTATCGTGGGGTTCG
CCGTCATGGCTTGATTATGGGTTGTTCAGTGACCGGTGTTAAACCTGCATCTGGAGATTCTCCTTTGCAGCTT
GGTCCAAAGGAAGAAGATGTTGAAAAGCCCGTTAATCCTTTTGTCTTTGCACTGCGCTTCGTTTTGGGTGCCT
TGGCAGCGACATGGTTTGTGTTGGTTCCTATTTACATGTGGCTGAAAGATCAAGTTGTTCCCAAAGGTCAGCC
AATCTAA (SEQ ID NO: 11)

>Protein translation *Glycine max* tMT2 - LIB3049-032-Q1-E1-G8

MGSVMLSGTEKLTLRTLTGNGLGFTGSDLHGKNFPRVSFAATTSAKVPNFRSIVVPKCSVSASRPSSQPRFIQ
HKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRNMIVVDVGGGTGFTTLGIVKHVDAKNVTILD
QSPHQLAKAKQKEPLKECKIIEGDAEDLPFRTDYADRYVSAGSIEYWPDPQRGIKEAYRVLKLGGKACLIGPV
YPTFWLSRFFADVWMLFPKEEEYIEWFQKAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPASGDSPLQL
GPKEEDVEKPVNPFVFALRFVLGALAATWFVLVPIYMWLKDQVVPKGQPI (SEQ ID NO: 25)

>CPR193236 *Allium Porrum* - LIB4521-015-Q1-K1-D6
ATGGCTTCCTCCATGCTCAGCGGAGCAGAAAGCCTCTCAATGCTCCGAATCCACCACCAACCCAAACTCACCT
TCTCGAGCCCATCCCTCCATTCCAAACCCACAAACCTCAAAATGGATCTCATCCCTTTCGCCACCAAGCATCA
AAAAACGAAAAAAGCTTCGATCTTTACATGCAGCGCGTCCTCATCATCCCGACCTGCTTCTCAGCCGAGGTTC
ATCCAGCACAAGCAGGAGGCGTTCTGGTTCTACAGGTTCCTGTCGATAGTGTACGACCATGTGATAAACCCAG
GGCACTGGACCGAGGACATGAGAGACGATGCGTTGGAGCCAGCCGAGCTGTACGATTCCAGGATGAAGGTGGT
GGACGTAGGAGGAGGAACTGGGTTCACCACCTTGGGGATTATAAAGCACATCGACCCTAAAAACGTTACGATT
CTGGATCAGTCTCCGCATCAGCTTGAGAAGGCTAGGCAGAAGGAGGCTTTGAAGGAGTGTACTATTGTTGAAG
GTGATGCTGAGGATCTCCCTTTTCCTACTGATACTTTCGATCGATATGTATCTGCTGGCAGCATAGAATACTG
GCCAGACCCACAAAGAGGGATAAAGGAAGCATACCGGGTTCTAAAACTGGGAGGCGTTGCCTGCTTGATAGGA
CCCGTGCACCCTACCTTCTGGCTTTCCAGGTTCTTCGCCGACATGTGGATGTTGTTCCCCCACCGAAGAAGAAT
ACATAGAGTGGTTTAAAAAGGCCGGGTTCAAAGATGTGAAGTTGAAGAGGATTGGCCCAAAATGGTACCGTGG
TGTGCGTAGACACGGGCTCATCATGGGCTGTTCCGTCACTGGTGTTAAACGTCTCTCTGGTGACTCCCCTCTT
CAGCTTGGACCGAAGGCGGAGGATGTGAAGAAGCCGATCAATCCATTCTCGTTCCTTCTGCGCTTCATTTTGG
GTACGATAGCAGCTACTTACTACGTTTTGGTGCCGATATACATGTGGATAAAGGATCAGATTGTACCGAAAGG
CCAGCCCATATGA (SEQ ID NO: 10)

>Protein translation *Allium Porrum* - LIB4521-015-Q1-K1-D6
MASSMLSGAESLSMLRIHHQPKLTFSSPSLHSKPTNLKMDLIPFATKHQKTKKASIFTCSASSSSRPASQPRF
IQHKQEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPAELYDSRMKVVDVGGGTGFTTLGIIKHIDPKNVTI
LDQSPHQLEKARQKEALKECTIVEGDAEDLPFPTDTFDRYVSAGSIEYWPDPQRGIKEAYRVLKLGGVACLIG
PVHPTFWLSRFFADMWMLFPTEEEYIEWFKKAGFKDVKLKRIGPKWYRGVRRHGLIMGCSVTGVKRLSGDSPL
QLGPKAEDVKKPINPFSFLLRFILGTIAATYYVLVPIYMWIKDQIVPKGQPI (SEQ ID NO: 24)

>CPR204065 *Gossypium hirsutum* tMT2 - LIB3272-054-P1-K1-C11
ATGGCTTCTTCCATGCTGAATGGAGCTGAAACCTTCACTCTCATCCGAGGTGTTACCCCAAAAAGTATTGGTT
TTTTGGGGTCAGGTTTACATGGGAAACAGTTTTCCAGTGCGGGTTTAATCTACAGTCCGAAGATGTCCAGGGT
AGGAACGACGATAGCCCCGAGGTGCAGCTTATCAGCGTCAAGGCCAGCTTCACAACCAAGATTCATACAACAC
AAAAAAGAGGCCTTTTGGTTCTACAGGTTCCTCTCAATTGTCTATGACCATGTCATAAACCCAGGTCACTGGA
CTGAAGACATGAGGGATGATGCACTTGAGCCGGCTGATCTCAATGACAGGGACATGGTAGTTGTAGATGTTGG
TGGTGGAACTGGTTTCACTACTTTGGGTATTGTTCAGCATGTGGATGCTAAGAATGTTACAATCCTTGACCAA
TCTCCTCACCAGCTTGCAAAGGCTAAACAGAAGGAGCCTCTCAAGGAATGCAACATAATTGAAGGTGATGCAG
AAGATCTTCCTTTTCCTACTGATTATGCCGATAGATATGTGTCTGCTGGAAGCATAGAGTACTGGCCAGACCC
ACAACGGGGGATCAAGGAAGCATACAGGGTGTTGAAACAAGGAGGAAAAGCTTGCTTAATTGGTCCTGTGTAC
CCTACATTTTGGTTGTCTCGTTTCTTTGCAGACGTTTGGATGCTTTTCCCTAAGGAGGAAGAATATATAGAGT
GGTTTGAAAAGGCTGGATTTAAGGATGTCCAACTCAAAAGGATTGGCCCTAAATGGTATCGTGGAGTTCGCCG
ACATGGTTTGATCATGGGGTGCTCTGTAACCGGTGTTAAACCCGCATCTGGGGACTCTCCTTTGCAGCTTGGA
CCTAAGGCAGAGGATGTATCAAAGCCGGTAAATCCGTTTGTATTTCTCTTACGCTTCATGTTGGGTGCCACTG
CAGCAGCATATTATGTACTGGTTCCTATCTACATGTGGCTCAAAGATCAAATTGTACCAGAGGGTCAACCAAT
CTAA (SEQ ID NO: 9)

>Protein translation *Gossypium hirsutum* tMT2 -LIB3272-054-P1-K1-C11
MASSMLNGAETFTLIRGVTPKSIGFLGSGLHGKQFSSAGLIYSPKMSRVGTTIAPRCSLSASRPASQPRFIQH
KKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRDMVVVDVGGGTGFTTLGIVQHVDAKNVTILDQ
SPHQLAKAKQKEPLKECNIIEGDAEDLPFPTDYADRYVSAGSIEYWPDPQRGIKEAYRVLKQGGKACLIGPVY
PTFWLSRFFADVWMLFPKEEEYIEWFEKAGFKDVQLKRIGPKWYRGVRRHGLIMGCSVTGVKPASGDSPLQLG
PKAEDVSKPVNPFVFLLRFMLGATAAAYYVLVPIYMWLKDQIVPEGQPI(SEQ ID NO: 23)

The protein sequence of tMT2 from *Arabidopsis thaliana* is compared against the tMT2 plant protein sequences listed above using BLASTP (Altschul *et al.*, *Nucleic Acids Res. 25*:3389-3402 (1997); see also www.ncbi.nlm.nih.gov/BLAST/). The calculated

protein identity of each sequence compared to the *Arabidopsis* sequence is shown in Figure 2. Also shown is a protein sequence alignment using the Pretty alignment program (Genetics Computer Group, Madison WI)(Figure 3).

Example 4

Preparation of constructs to direct the expression of the wild type tMT2 and mutant tMT2 gene sequences of *Arabidopsis thaliana* and tMT2 gene sequences from other crop plant species in a prokaryotic expression system.

A computer program is used to predict the chloroplast targeting peptide cleavage site of the plant tMT2 protein ("ChloroP", Center for Biological Sequence Analysis, Lyngby, Denmark). The result of the search is as follows:

Name	Length	Score	cTP	CS-score	cTP-length
Arabidopsis	338	0.585	Y	6.467	51

Based on this information, the tMT2 protein from *Arabidopsis thaliana,* ecotype Landsberg is engineered to remove the predicted chloroplast target peptide to allow for the expression of the mature protein in *E. coli.* In order for these proteins to be expressed in a prokaryotic expression system, an amino terminal methionine is required. To make the addition of a 5' ATG the tMT2 coding sequence is amplified from cDNA of wild type and the high δ-tocopherol hdt6, and hdt16 mutant lines of *Arabidopsis thaliana*, ecotype Columbia, and the high δ-tocopherol hdt2 and hdt10 mutant lines of *Arabidopsis thaliana,* ecotype Landsberg.

PolyA^+ RNA is isolated from each source using an adapted biotin/streptavadin procedure based on the "mRNA Capture Kit" by Roche Molecular Biochemicals (Indianapolis, IN). A young plantlet, approximately 1 cm tall, with root tissue removed is homogenized in CTAB buffer (50mM Tris-HCl pH 9, 0.8M NaCl, 0.5% CTAB, 10mM EDTA), extracted with chloroform, and pelleted with centrifugation. As specified by the manufacturer's instructions, polyA^+ RNA in the soluble fraction is hybridized to biotin-labeled oligo-dT, immobilized on streptavadin-coated PCR tubes and washed. The first strand cDNA is synthesized using the "1st strand cDNA synthesis kit for RT-PCR" (Roche Molecular Biochemicals) in a 50μl volume according to the manufacturer's protocol. Following the cDNA synthesis, the soluble contents of the tube are replaced with equal volume amplification reaction mixture. The components of the mixture at final concentration consist of:

- 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular Biochemicals)
- 200μM dNTPs
- 300nM each synthetic oligonucleotide primers;

  #17180 FORWARD-NcoI

  5'GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCTACTAGATGCAGCAGCAGCAGC 3' (SEQ ID NO: 79) and

  #17181 REVERSE-Sse8387I

  5'GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG 3'. (SEQ ID NO: 78)

  Each primer contains regions for GATEWAY™ cloning (Life Technologies Division, Invitrogen Corporation) as well as conventional restriction enzyme sites.
- 0.4μl Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals)

Constructs are also prepared to direct expression of the engineered *Brassica napus, Oryza sativa, Zea mays, Glycine max, Allium Porrum, and Gossypium hirsutum* tMT2 sequences in a prokaryotic expression vector. The mature protein coding region of each tMT2 with the aminoterminal methionine, as described above, is amplified from plasmid DNA using the following oligonucleotide primers in the polymerase chain reaction.

The mature *Brassica napus* tMT2 coding sequence is amplified from LIB4153-013-R1-K1-B7 (SEQ ID NO: 13) using the synthetic oligonucleotide primers:

Brassica forward (17509)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCGACAAGATGCAGCAGCAGCAGCAG (SEQ ID NO: 77).

Brassica reverse (17181)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG (SEQ ID NO: 78).

The mature *Oryza sativa* tMT2 coding sequence is amplified from LIB4371-041-R1-K1-F7 (SEQ ID NO: 12) using the synthetic oligonucleotide primers:

Rice forward (17512)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGCGGCTGAGGTGCGCGGCGTCGTCG

(SEQ ID NO: 79).

Rice reverse (17513)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATCGGCATGCCTTTGGGCAC (SEQ ID NO: 80).

The mature Zea *mays* tMT2 coding sequence is amplified from LIB3587-273-Q1-K6-C5 (SEQ ID NO: 8) using the synthetic oligonucleotide primers:

Corn forward (17510)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGAGGCTGCGATGCGCGGCGTCGTCG (SEQ ID NO: 81).

Corn reverse (17511)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATTGGCATGCCTTTTGGCACG (SEQ ID NO: 82).

The mature *Glycine max* tMT2 coding sequence is amplified from LIB3049-032-Q1-E1-G8 (SEQ ID NO: 11) using the synthetic oligonucleotide primers:

Soy forward (17516)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGTACCCAAGTGTAGTGTCTCGGC (SEQ ID NO: 83).

Soy reverse (17517)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATTGGCTGACCTTTGGGAAC (SEQ ID NO: 84).

The mature *Allium Porrum* tMT2 coding sequence is amplified from LIB4521-015-Q1-K1-D6 (SEQ ID NO: 10) using the synthetic oligonucleotide primers:

Leek forward (17518)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGATCTTTACATGCAGCGCGTCCT (SEQ ID NO: 85).

Leek reverse (17519)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCATATGGGCTGGCCTTTCGGTAC (SEQ ID NO: 86).

The mature *Gossypium hirsutum* tMT2 coding sequence is amplified from LIB3272-054-P1-K1-C11 (SEQ ID NO: 9) using the synthetic oligonucleotide primers:

Cotton forward (17514)

GGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGCCCCGAGGTGCAGCTTATCAGCG

(SEQ ID NO: 87).

Cotton reverse (17515)

GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTTAGATTGGTTGACCCTCTGGTAC (SEQ ID NO: 88).

The components of each 100µl PCR reaction at final concentration consisted of:

- 0.5µl plasmid DNA diluted 1:20 with water
- 1X Buffer 2 (Expand™ High Fidelity PCR System, Roche Molecular Biochemicals)
- 200µM dNTPs
- 300nM each, synthetic oligonucleotide primers
- 0.8µl Expand™ High Fidelity Polymerase (Roche Molecular Biochemicals)

The tMT2 gene from each source is PCR amplified for 30 cycles using the following "touchdown" cycling profile. For each reaction the reaction mix is pre-incubated for 5 minutes at 95°C, during which the polymerase is spiked in. The product is then amplified for 15 cycles, each cycle consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 1.5 minutes. The annealing temperature is decreased by 1°C per cycle for each of the previous 15 cycles. An additional 15 cycles follow, consisting of 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 1.5 minute, followed by a 7 minute hold at 72°C. The resulting amplification product is visualized as a clean band of the appropriate size for each species on a 0.8% agarose gel.

The resulting PCR products are subcloned into pDONR™201 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD).

To verify that no errors are introduced by the PCR amplification, the double stranded DNA sequence is obtained using standard sequencing methodology. The tMT2 sequences are then recombined behind the T7 promoter in the prokaryotic expression vector pET-DEST42 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD).

The following sequences represent the mature amino acid sequences of the wild type and mutant genes which may be expressed in *E. coli*, following the addition of an amino terminal methionine. The bolded and italicized amino acid residues represent the location of the substitution in each of the mutants.

Mature wildtype Arabidopsis tMT2 protein as expressed *in E. coli*:
ATRCSSSSVSSSRPSAQPRFIQHKKEAYWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLSHPDMRVVDVGG
GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
GLIMGCSVTGVKPASGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
(SEQ ID NO: 28)

Mature mutant hdt2 Arabidopsis tmt2 protein as expressed *in E. coli*
ATRCSSSSVSSSRPSAQPRFIQHKKEAYWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLSHPDMRVVDVGG
GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
GLIMGCSVTGVKPASGDSPLQLGPKE***K***DVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
(SEQ ID NO: 29)

Mature mutant hdt6 Arabidopsis tmt2 protein as expressed *in E. coli*
ATRCSSSSVSSSRPSAQPRFIQHKK***K***AYWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLSHPDMRVVDVGG
GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
GLIMGCSVTGVKPASGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
(SEQ ID NO: 30)

Mature mutant hdt10 Arabidopsis tmt2 protein as expressed *in E. coli*
ATRCSSSSVSSSRPSAQPRFIQHKKEAYWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLSHPDMRVV***N***VGG
GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
GLIMGCSVTGVKPASGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
(SEQ ID NO: 31)

Mature mutant hdt16 Arabidopsis tmt2 protein as expressed *in E. coli*
ATRCSSSSVSSSRPSAQPRFIQHKKEAYWFYRFLSIVYDHVINPGHW***I***EDMRDDALEPADLSHPDMRVVDVGG
GTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDPQ
RGIREAYRVLKIGGKACLIGPVYPTFWLSRFFSDVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRRH
GLIMGCSVTGVKPASGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQPI
(SEQ ID NO: 32)

Mature *Brassica napus* tMT2 as expressed *in E. coli*
ATRCSSSSSVSASRPSAQPRFIQHKKEAYWFYRFLSIVYDHIINPGHWTEDMRDDALEPADLSHPDMRVVDVG
GGTGFTTLGIVKTVKAKNVTILDQSPHQLAKAKQKEPLKECKIVEGDAEDLPFPTDYADRYVSAGSIEYWPDP
QRGIREAYRVLKIGGKACLIGPVHPTFWLSRFFADVWMLFPKEEEYIEWFKNAGFKDVQLKRIGPKWYRGVRR
HGLIMGCSVTGVKPASGDSPLQLGPKEEDVEKPVNNPFSFLGRFLLGTLAAAWFVLIPIYMWIKDQIVPKDQP
I. (SEQ ID NO: 33)

Mature *Oryza sativa* tMT2 as expressed *in E. coli*
RLRCAASSSSAAARPMSQPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLYSRKLRVVDVG
GGTGFTTLGIVKRVDPENVTLLDQSPHQLEKAREKEALKGVTIMEGDAEDLPFPTDTFDRYVSAGSIEYWPDP
QRGIKEAYRVLRLGGVACMIGPVHPTFWLSRFFADMWMLFPKEEEYIEWFKKAGFKDVKLKRIGPKWYRGVRR
HGLIMGCSVTGVKREHGDSPLQLGPKVEDVSKPVNPITFLFRFLMGTICAAYYVLVPIYMWIKDQIVPKGMPI
. (SEQ ID NO: 34)

Mature *Zea mays* tMT2 as expressed *in E. coli*
RLRCAASSSPAAARPATAPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLFSRHLTVVDVG
GGTGFTTLGIVKHVNPENVTLLDQSPHQLDKARQKEALKGVTIMEGDAEDLPFPTDSFDRYISAGSIEYWPDP
QRGIKEAYRVLRFGGLACVIGPVYPTFWLSRFFADMWMLFPKEEEYIEWFKKAGFRDVKLKRIGPKWYRGVRR

HGLIMGCSVTGVKRERGDSPLELGPKAEDVSKPVNPITFLFRFLVGTICAAYYVLVPIYMWIKDQIVPKGMPI
(SEQ ID NO: 35)

Mature *Glycine max* tMT2 as expressed *in E. coli*
VPKCSVSASRPSSQPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRNMIVVDVGGGTG
FTTLGIVKHVDAKNVTILDQSPHQLAKAKQKEPLKECKIIEGDAEDLPFRTDYADRYVSAGSIEYWPDPQRGI
KEAYRVLKLGGKACLIGPVYPTFWLSRFFADVWMLFPKEEEYIEWFQKAGFKDVQLKRIGPKWYRGVRRHGLI
MGCSVTGVKPASGDSPLQLGPKEEDVEKPVNPFVFALRFVLGALAATWFVLVPIYMWLKDQVVPKGQPI
(SEQ ID NO: 36)

Mature *Allium Porrum* as expressed *in E. coli*
IFTCSASSSSRPASQPRFIQHKQEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPAELYDSRMKVVDVGGGT
GFTTLGIIKHIDPKNVTILDQSPHQLEKARQKEALKECTIVEGDAEDLPFPTDTFDRYVSAGSIEYWPDPQRG
IKEAYRVLKLGGVACLIGPVHPTFWLSRFFADMWMLFPTEEEYIEWFKKAGFKDVKLKRIGPKWYRGVRRHGL
IMGCSVTGVKRLSGDSPLQLGPKAEDVKKPINPFSFLLRFILGTIAATYYVLVPIYMWIKDQIVPKGQPI
(SEQ ID NO: 37).

Mature *Gossypium hirsutum* tMT2 as expressed *in E. coli*
APRCSLSASRPASQPRFIQHKKEAFWFYRFLSIVYDHVINPGHWTEDMRDDALEPADLNDRDMVVVDVGGGTG
FTTLGIVQHVDAKNVTILDQSPHQLAKAKQKEPLKECNIIEGDAEDLPFPTDYADRYVSAGSIEYWPDPQRGI
KEAYRVLKQGGKACLIGPVYPTFWLSRFFADVWMLFPKEEEYIEWFEKAGFKDVQLKRIGPKWYRGVRRHGLI
MGCSVTGVKPASGDSPLQLGPKAEDVSKPVNPFVFLLRFMLGATAAAYYVLVPIYMWLKDQIVPEGQPI
(SEQ ID NO: 38)

Example 5.

A 2-methylphytylplastoquinol methyltransferase enzymatic assay is performed on the mature cloned genes expressed in *E. coli* to test for functionality of the encoded proteins.

A culture is started by inoculating 100 mL of LB media with appropriate antibiotics with an overnight starter culture of *E. coli* BL21(DE3) cells that is previously transformed with prokaryotic expression constructs described in Example 4. The initial inoculation results in an optical density of $OD_{600} = 0.1$ and the culture is grown at 25°C to a final density of $OD_{600} = 0.6$. An amount corresponding to a final concentration of 0.4 mM IPTG is added to induce protein expression, and the cells are then incubated at 25°C for 3 hours until harvest.

The cells are chilled on ice for 5 minutes and then spun down at 5000 *x* g for 10 minutes. The cell pellet is stored at -80°C overnight after thoroughly aspirating off the supernatant.

The cell pellet is thawed on ice and resuspended in 4mL of extraction buffer XB (10mM HEPES-KOH pH7.8, 5mM DTT, 1mM AEBSF, 0.1 mM aprotinin, 1mg/ml leupeptin). Cells are disrupted using a French press by making two passes through the pressure cell at 20,000psi. Triton X-100 is added to a final concentration of 1% and the extract is incubated on ice for one hour. The cell homogenate is then centrifuged at 5000 *x* g for 10 minutes at 4°C.

The enzyme assays are run on the same day that the cells are extracted. The assays are run in 10mL polypropylene culture tubes with a final volume of 1mL. A reaction mixture consisting of the following is prepared and brought to a final volume of 950 μL with distilled water.

Reaction mixture:

50mM Tris-HCl pH 8.0

5mM dithiothreitol (DTT, 100mM stock solution in water)

100μM 2-methylphytylplastoquinol (404g/mol)

0.5% Tween 80 (added directly to phytylplastoquinol after evaporating off solvent)

1.7μM ^{14}C-SAM (58μCi/μmole)

2-Methyl-phytylplastoquinol and 2-methyl-geranylgeranylplastoquinol are synthesized as follows:

Fresh BF_3-etherate (0.3ml) is added drop by drop to a solution of 400 mg methylquinol, 1000 mg isophytol in 10 ml dry dioxane. The mixture is stirred under N_2 in the dark and is maintained at 50°C for 2 hours. The reaction mixture is hydrolyzed with ice, extracted with 3x15 ml petroleum ether/diethyl ether (1:1), the extract is washed several times with water to remove unused methylquinol, and dried with $MgSO_4$. The solvent is evaporated off with a rotavapor to yield an oil like crude reaction product containing a mixture of methylplastoquinols. At this stage the reaction mixture is either separated into various methylphytylplastoquinols by flash chromatography followed by HPLC purification or alternatively oxidized to yield the more stable methylplastoquinones. This is achieved by addition of a small amount of Ag_2O (200 mg) to the reaction product dissolved in diethyl ether for 1 hour. Removal of the Ag_2O by filtration provides the methylphytylplastoquinone mixture.

The synthesis of methylphytylplastoquinol as described above gives six isomers, namely 2'-cis and 2'-trans isomers of 2-methyl-3-phytylplastoquinol, 2-methyl-5-phytylplastoquinol 2-methyl-6-phytylplastoquinol. Purification of the six isomers is achieved by an initial separation of the methylphytylplastoquinol mixture into two bands on TLC (PSC-Fertigplatten Kieselgel 60 $F_{254+366}$, Merck, Darmstadt), using solvent system petroleum ether:diethyl ether (7:3). The final purification of isomers of methylplastoquinols is achieved by semi-preparative HPLC.

HPLC is performed on a HP1100 series HPLC system consisting of HP G1329A Auto Sampler, HP G1311A Quaternary Pump, HP G1315A Diode Array Detector, HP G1321A Fluorescence Detector. Excitation is performed at 290 nm, emission is measured at 336 nm. In parallel, absorption is measured using a diode array detector set at 210 and 254 nm. The flow rate is kept at 5 mL/min. Plastoquinols are separated on isocratic HPLC using 90% Hexane:Methyl-Tertbutyl-Ether (90:10) on an Agilent Zorbax Silica 9.4 X 250 mm column.

Synthesis of 2-methyl-6-geranylgeranylplastoquinol is performed as the synthesis of 2-methyl-6-phytylplastoquinol, except geranyllinalool is used instead of isophytol for synthesis. The pure product is obtained from flash chromatography followed by repetitive TLC as described above.

To perform the methyltransferase assay 50μL of the cell extract is added to the assay mixture and mixed well. The reaction is initiated by adding ^{14}C-SAM (ICN) and incubating for one hour at 30°C in the dark. The reactions are then transferred to 15mL glass screw cap tubes equipped with Teflon coated caps. The reaction mixture is extracted with 4mL 2:1 $CHCl_3$/MeOH with 1mg/mL butylated hydroxy toluene (BHT) and mixed by vortex for 30 seconds. The tubes are centrifuged for 5 minutes to separate layers and the organic phase (bottom) is transferred to fresh 15mL glass tube. The $CHCl_3$ is evaporated off under a stream of nitrogen gas at 37°C for about 15 minutes. The residue is dissolved in 200 μL of EtOH containing 1% pyrogallol and then mixed by vortex for 30 seconds. The resuspension is filtered into a brown LC vial equipped with an insert and analyzed by HPLC using a normal phase column (Agilent 4.6 x 250 mm Zorbax Sil, Agilent Technologies). The elution program is an isocratic flow of 10% methyl-*tert*-butyl-ether (MTBE) in hexane at 1.5 ml/minute for 12 minutes. Prior to each injection, a clean up run of 75% MTBE in hexane for 3 minutes is done, followed by a re-equilibration step of 10% MTBE in hexane for 3 minutes.

As a positive control, a pea chloroplast concentrate, which is known to have tMT2 activity, is prepared according to the procedure described by Arango and Heise, *Biochem J. 336*:531-533 (1998).

The results of these enzyme assays are shown in Figures 4-8. The series of HPLC chromatograms demonstrate that the cells transformed with the MT1 from *Anabaena*, which is known to have tMT2 activity (Figure 4) and the tMT2 from *Arabidopsis* (Figure

5) accumulate methylated products comigrating with a 2,3-dimethyl-5-phytylplastoquinone standard. The mutated tMT2 gene from *Arabidopsis* (hdt2) accumulated significantly less methylated products (Figure 6) than the wildtype tMT2 gene (Figure 5), showing that it has a decreased tMT2 activity. By way of comparison, the negative control where substrate is withheld from the cells transformed with the MT1 from *Anabaena* did not show a significant peak corresponding to the methylated products (Figure 7). Furthermore, the positive control of pea chloroplasts showed peaks corresponding to the methylated products obtained in the assays using *E. coli* extracts from strains harboring the MT1 and tMT2 expression constructs (Figure 8).

Expression and enzyme assay of crop tMT2 orthologs

tMT2 orthologs from *Brassica* (pMON67233), corn (pMON67234), leek (pMON67235), soybean (pMON67245), rice (pMON67232), and cotton (pMON67244), as well as the wild type *Arabidopsis* tMT2 (pMON67191), the hdt2 mutant (pMON67207), and the hdt10 mutant (pMON67243) are expressed as mature proteins in *E. coli* (Example 4). An *Anabaena* hdt2 otholog is expressed from pMON67190. The *Anabaena* MT1 (pMON67174) and empty vector (pMON67179) are used as positive and negative controls, respectively. Cell growth, cell harvest, cell disruption, and enzyme assay are performed as described in Example 5. HPLC-purified 2-methyl-6-phytylplastoquinol is used as methyl group acceptor.

Table 3: 2-Methyl-6-phytylplastoquinol activity of recombinant expressed tMT2 genes

pMON #	Gene	Enzyme activity [μU/mg protein]
67174	*Anabaena* MT1	6.5
67179	Plasmid control	< 1
67190	*Anabaena* tMT2 ortholog	< 1
67191	*Arabidopsis* tMT2	10
67207	*Arabidopsis* hdt2 mutant	1.1
67232	Rice tMT2 ortholog	4
67233	*Brassica* tMT2 ortholog	2
67234	Corn tMT2 ortholog	< 1
67235	Leek tMT2 ortholog	< 1
67243	*Arabidopsis* hdt10 mutant	< 1
67244	Cotton tMT2 ortholog	23.4
67245	Soy tMT2 ortholog	16.8

E. coli extracts expressing the *Anabaena* MT1, as well as mature proteins of the *Arabidopsis* tMT2, rice tMT2, cotton tMT2, and the soybean tMT2 are assayed as described in Example 5 using HPLC-purified 2-methyl-6-phytylplastoquinol, 2-methyl-5-phytylplastoquinol, or 2-methyl-3-phytylplastoquinol as methyl group acceptor. The assay demonstrates that tMT2 orthologs have a broader substrate range than the bacterial MT1 (Fig. 24).

Methyltransferase assays are performed using cell free *E. coli* extracts used in the experiments described above, expressing the *Anabaena* MT1, as well as the mature *Arabidopsis*, rice, cotton, and soybean tMT2s and 2-methyl-6-gernanylplastoquinol, δ-tocopherol, γ-tocopherol, or β-tocopherol as methyl group accepting substrates. Enzyme activities are below the limit of detection with all four substrates.

Example 6:

Transformation and expression of a wild type *Arabidopsis* tMT2 gene in *Arabidopsis thialiana*.

The coding region of tMT2 is amplified from the EST clone Lib 3177-021-P1-K1-A3 (SEQ ID NO: 1) using the synthetic oligonucleotide primers;

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#17286 FORWARD
GGGGACAAGTTTGTACAAAAAAGCAGGCTGCGGCCGCTGAACAATGGCCTCTTTGATGCTCAACG (SEQ ID NO: 89) and
# 17181 REVERSE
GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCAGGTCAGATGGGTTGGTCTTTGGGAACG (SEQ ID NO: 90).
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The amplification reaction consists of 1.0μl of EST template, 2.5 μl 20X dNTPs, 2.5 μl of each oligonucleotide primers, 5 μl 10X PCR buffer, 35.75 μl H20 and 0.75 μl Expand High Fidelity DNA Polymerase. PCR conditions for amplification are as follows:

- 1 cycle of 94° for 2 minutes, 10 cycles of 94°–15 seconds; 55°–30 seconds; and 72°– 1.5 minutes,
- 15 cycles of 94°–15 seconds; 55°–30 seconds; and 72°– 1.5 minutes adding 5 seconds to the 72° extension with each cycle,
- 1 cycle of 72° for 7 minutes.

After amplification, the samples are purified using a Qiagen PCR cleanup column (Qiagen Company, Valencia, California), suspended in 30 μl water. The PCR reaction

products are separated on an agarose gel and visualized according to standard methodologies. The resulting PCR products are subcloned into pDONR™201 (Life Technologies, A Division of Invitrogen Corp., Rockville, MD) using the GATEWAY cloning system (Life Technologies, A Division of Invitrogen Corp., Rockville, MD). The resultant intermediate plasmid is named pMON67204 and the tMT2 sequence is confirmed by DNA sequencing using standard methodologies.

The wild type *Arabidopsis* tMT2 sequence is then cloned from the pMON67204 donor vector into the pMON67150 destination vector using the GATEWAY Technology kit (Life Technologies, a Division of Invitrogen Corporation, Rockville, MD.) according to the manufacturer's instructions. This destination vector is a GATEWAY compatible binary vector containing the napin cassette derived from pCGN3223 (described in U.S. Patent No. 5,639,790). The resultant expression vector is named pMON67205 (Figure 9) and is used to drive the expression of the tMT2 sequence in seeds.

The plant binary construct described above is used in *Arabidopsis thaliana* plant transformation to direct the expression of the tMT2 gene in the embryo. The binary vector construct is transformed into ABI strain *Agrobacterium* cells by the method of Holsters *et al. Mol. Gen. Genet. 163*:181-187 (1978). Transgenic *Arabidopsis thaliana* plants are obtained by *Agrobacterium*-mediated transformation of *Arabidopsis* wild type and the high δ-tocopherol mutants hdt2, hdt10, and hdt16 as described by Valverkens *et al., Proc. Nat. Acad. Sci. 85*:5536-5540 (1988), Bent *et al., Science* 265:1856-1860 (1994), and Bechtold *et al., C.R. Acad. Sci., Life Sciences* 316:1194-1199 (1993). Transgenic plants are selected by sprinkling the transformed T_1 seeds directly onto soil and then vernalizing them at 4°C in the absence of light for 4 days. The seeds are transferred to 21°C, 16 hours light and sprayed with a 1:200 dilution of Finale (AgrEvo Environmental Health, Montvale, NJ) at 7 days and 14 days after seeding. Transformed plants are grown to maturity and the T_2 seed that is produced is analyzed for tocopherol content. The resulting tocopherol data shown in Tables 4 and 5 confirm a reduction of δ-tocopherol in favor of γ and α-tocopherol production in the high δ-tocopherol mutants and in wild type *Arabidopsis* lines. Tables 4 and 5 contain the results of HPLC analysis using the methodology (with minor

modifications) described in Savidge et al., *Plant Phys.* 129:321-332 (2000), Isolation and Characterization of Homogentisate Phytltransferase Genes from *Synechocystis* sp PCC 6803 and *Arabidopsis*.

Table 4 below details the results of the T_2 seed analysis.

Table 4

ng alpha toco./mg seed	ng beta toco./mg seed	ng gamma toco./mg seed	ng delta toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Line #		% Delta	Average % Delta
5.88	0.00	529.64	18.87	554.39	69000076011	9979-AT00002-81:@.0001.	1		3.4	3.2
5.45	0.00	525.89	17.44	548.78	69000076009	9979-AT00002-81:@.0004.	4		3.2	
5.74	0.00	511.61	16.32	533.67	69000075994	9979-AT00002-81:@.0003.	3		3.1	
5.04	0.00	507.38	16.10	528.52	69000076023	9979-AT00002-81:@.0002.	2		3.0	
7.74	0.00	466.14	11.53	485.41	69000075463	67205-AT00002:0010.	10	T2	2.4	1.2
8.76	0.00	460.36	7.00	476.12	69000075540	67205-AT00002:0001.	1	T2	1.5	
8.33	0.00	445.02	6.71	460.06	69000075564	67205-AT00002:0004.	4	T2	1.5	
8.46	0.00	443.94	6.67	459.06	69000075502	67205-AT00002:0014.	14	T2	1.5	
11.13	0.00	447.27	6.35	464.75	69000075526	67205-AT00002:0016.	16	T2	1.4	
9.07	0.00	470.64	6.49	486.19	69000075552	67205-AT00002:0003.	3	T2	1.3	
8.10	0.00	422.89	5.82	436.81	69000075538	67205-AT00002:0002.	2	T2	1.3	
8.64	0.00	473.01	6.47	488.12	69000075603	67205-AT00002:0008.	8	T2	1.3	
9.25	0.00	488.63	6.43	504.32	69000075590	67205-AT00002:0007.	7	T2	1.3	
7.71	0.00	475.80	6.21	489.72	69000075588	67205-AT00002:0006.	6	T2	1.3	
7.77	0.00	458.67	5.71	472.15	69000075475	67205-AT00002:0011.	11	T2	1.2	
8.85	0.00	455.97	5.59	470.41	69000075576	67205-AT00002:0005.	5	T2	1.2	
10.27	0.00	349.67	3.05	362.98	69000075514	67205-AT00002:0015.	15	T2	0.8	
9.22	0.00	371.75	2.84	383.81	69000075499	67205-AT00002:0013.	13	T2	0.7	
8.68	0.00	348.97	2.53	360.18	69000075451	67205-AT00002:0009.	9	T2	0.7	
7.96	0.00	413.19	2.40	423.55	69000075487	67205-AT00002:0012.	12	T2	0.6	
7.00	0.00	277.36	286.49	570.84	69000077835	hdt2:@.0001.	1		50.2	49.7
6.57	0.00	273.89	278.92	559.38	69000077809	hdt2:@.0004.	4		49.9	
6.90	0.00	277.90	279.96	564.77	69000077811	hdt2:@.0003.	3		49.6	
6.93	0.00	275.20	273.89	556.01	69000077823	hdt2:@.0002.	2		49.3	
8.35	0.00	365.85	143.68	517.88	69000075639	67205-hdt2:0011.	11	T2	27.7	20.5
7.75	0.00	384.44	127.60	519.79	69000075689	67205-hdt2:0016.	16	T2	24.5	
7.05	0.00	358.91	105.17	471.13	69000075627	67205-hdt2:0010.	10	T2	22.3	
8.33	0.00	342.11	98.01	448.45	69000075665	67205-hdt2:0014.	14	T2	21.9	

ng alpha toco./mg seed	ng beta toco./mg seed	ng gamma toco./mg seed	ng delta toco./mg seed	ng total toco./mg seed	Serial Number	Pedigree	Line #		% Delta	Average % Delta
6.73	0.00	410.18	112.97	529.88	69000075716	67205-hdt2:0006.	6	T2	21.3	
6.89	0.00	357.86	98.47	463.22	69000075704	67205-hdt2:0007.	7	T2	21.3	
6.85	0.00	352.48	96.71	456.04	69000075691	67205-hdt2:0008.	8	T2	21.2	
8.06	0.00	356.89	96.10	461.05	69000075754	67205-hdt2:0002.	2	T2	20.8	
7.60	0.00	311.53	82.55	401.68	69000075677	67205-hdt2:0015.	15	T2	20.6	
7.81	0.00	344.03	88.44	440.28	69000075615	67205-hdt2:0009.	9	T2	20.1	
7.50	0.00	368.30	88.66	464.46	69000075641	67205-hdt2:0012.	12	T2	19.1	
7.13	0.00	336.24	80.34	423.71	69000075728	67205-hdt2:0005.	5	T2	19.0	
7.78	0.00	345.26	81.26	434.30	69000075766	67205-hdt2:0001.	1	T2	18.7	
8.82	0.00	340.61	72.71	422.15	69000075730	67205-hdt2:0004.	4	T2	17.2	
8.11	0.00	418.69	81.01	507.81	69000075742	67205-hdt2:0003.	3	T2	16.0	
6.08	0.00	365.54	69.78	441.40	69000075653	67205-hdt2:0013.	13	T2	15.8	
3.36		262.76	180.18	446.30	69000157140	hdt16:@.0007.	Control	M5	40.4	38.2
3.36		290.12	177.76	471.24	69000157114	hdt16:@.0003.	Control	M5	37.7	
2.54		305.52	178.20	486.25	69000157099	hdt16:@.0005.	Control	M5	36.6	
4.93		248.24	67.78	320.95	69000156403	AT_G119:@.	PMON67205	R2	21.1	16.0
3.55		232.71	62.01	298.26	69000156667	AT_G36:@.	PMON67205	R2	20.8	
5.55		282.81	64.06	352.42	69000156679	AT_G37:@.	PMON67205	R2	18.2	
6.79		273.40	55.90	336.09	69000156617	AT_G31:@.	PMON67205	R2	16.6	
5.65		377.29	52.27	435.22	69000156631	AT_G33:@.	PMON67205	R2	12.0	
5.82		256.67	20.04	282.53	69000156655	AT_G35:@.	PMON67205	R2	7.1	
4.32		356.41	71.85	432.59	69000157037	hdt10:@.0001.	Control	M6	16.6	9.6
5.73		469.11	12.79	487.62	69000157049	hdt10:@.0002.	Control	M6	2.6	
3.39		308.41	27.44	339.24	69000156528	AT_G22:@.	PMON67205	R2	8.1	2.9
5.53		350.19	28.83	384.55	69000156592	AT_G29:@.	PMON67205	R2	7.5	
4.33		329.32	23.29	356.94	69000156489	AT_G18:@.	PMON67205	R2	6.5	
5.20		344.82	19.81	369.84	69000156566	AT_G26:@.	PMON67205	R2	5.4	
6.14		348.51	19.38	374.03	69000156453	AT_G15:@.	PMON67205	R2	5.2	
5.12		394.47	14.59	414.19	69000156578	AT_G27:@.	PMON67205	R2	3.5	
7.01		473.37	13.03	493.40	69000156530	AT_G23:@.	PMON67205	R2	2.6	
6.82		355.34	3.94	366.10	69000156580	AT_G28:@.	PMON67205	R2	1.1	
4.41		395.46	3.82	403.69	69000156477	AT_G17:@.	PMON67205	R2	0.9	
4.64		383.13	2.46	390.23	69000156542	AT_G24:@.	PMON67205	R2	0.6	
6.21		319.67	1.91	327.79	69000156465	AT_G16:@.	PMON67205	R2	0.6	
4.79		291.39	1.59	297.77	69000156441	AT_G14:@.	PMON67205	R2	0.5	
4.72		393.79	1.89	400.40	69000156491	AT_G19:@.	PMON67205	R2	0.5	
5.97		378.05	1.59	385.62	69000156516	AT_G21:@.	PMON67205	R2	0.4	
6.16		358.64	0.00	364.80	69000156554	AT_G25:@.	PMON67205	R2	0.0	

mp: indicates "metabolic profiling".

Table 5 below depicts the results of the analysis of T3 seed data from pMON67205 in hdt2 mutant lines.

Table 5

Crop	Biotype	Serial Number	mp:aT	mp:gT	mp:dT	total toco.	% delta	Gen	Pedigree	Construct
AT	SEED	69000357524	2	280	190	472	40.3	M7	hdt2:@.0001.0001.	
AT	SEED	69000357512	3	262	208	473	44.0	M7	hdt2:@.0001.0002.	
AT	SEED	69000357625	4	263	204	471	43.3	M7	hdt2:@.0001.0003.	
AT	SEED	69000357613	4	271	220	495	44.4	M7	hdt2:@.0001.0004.	
AT	SEED	69000357803	6	436	26	468	5.6	R3	67205-hdt2:0003.0001.	67205
AT	SEED	69000357790	4	336	149	489	30.5	R3	67205-hdt2:0003.0002.	67205
AT	SEED	69000357788	4	332	112	448	25.0	R3	67205-hdt2:0003.0003.	67205
AT	SEED	69000357776	3	334	140	477	29.4	R3	67205-hdt2:0003.0004.	67205
AT	SEED	69000357764	4	324	128	456	28.1	R3	67205-hdt2:0003.0005.	67205
AT	SEED	69000357598	3	363	97	463	21.0	R3	67205-hdt2:0003.0006.	67205
AT	SEED	69000357586	4	339	145	488	29.7	R3	67205-hdt2:0003.0007.	67205
AT	SEED	69000357574	4	372	99	475	20.8	R3	67205-hdt2:0003.0008.	67205
AT	SEED	69000357562	5	388	72	465	15.5	R3	67205-hdt2:0003.0009.	67205
AT	SEED	69000357550	4	341	63	408	15.4	R3	67205-hdt2:0013.0001.	67205
AT	SEED	69000357548	3	352	60	415	14.5	R3	67205-hdt2:0013.0002.	67205
AT	SEED	69000357536	4	386	54	444	12.2	R3	67205-hdt2:0013.0003.	67205
AT	SEED	69000358209	4	381	54	439	12.3	R3	67205-hdt2:0013.0004.	67205
AT	SEED	69000358196	6	413	73	492	14.8	R3	67205-hdt2:0013.0005.	67205
AT	SEED	69000358184	3	379	62	444	14.0	R3	67205-hdt2:0013.0006.	67205
AT	SEED	69000358172	5	382	63	450	14.0	R3	67205-hdt2:0013.0007.	67205
AT	SEED	69000358160	5	359	49	413	11.9	R3	67205-hdt2:0013.0008.	67205
AT	SEED	69000357601	4	371	4	379	1.1	R3	67205-hdt2:0013.0009.	67205

Example 7:

Method to prepare double gene constructs for expression in soybean and *Arabidopsis*.

Constructs are made containing promoters that provide seed-specific expression of the tMT2 gene alone and in combination with the GMT gene in soybean. Additionally the tMT2 gene is cloned behind the napin promoter and cloned into a binary vector with the HPT gene from *Arabidopsis* and in another double gene construct with the prenyltransferase (PT) gene (*slr*1736) from *Synechocystis* (pMON67224 and pMON67223 as shown in Figures 14 and 15, respectively).

Soybean Constructs

The wild type *Arabidopsis* tMT2 gene is cloned in between the 7S promoter and the pea SSU Rubisco 3' UTR in the vector pCGN3892 to create pMON67220 (Figure 10). This clone is then digested with Not I and the expression cassette is subcloned into the

plant binary expression vector pCGN11121 to create pMON67226 (Figure 11). This construct is used to transform soybean. Additionally, the *Arabidopsis* GMT between the 7S promoter and the pea SSU Rubisco 3' UTR is cut out from pMON36503 and then cloned into pMON67220 to create pMON67225 (Figure 12). These two genes under the control of 7S promoters are then cut out of pMON67225 with NotI and cloned into the Not site of pCGN11121 to create pMON67227 (Figure 13). This double gene construct is then used to transform soybean according to the procedure set forth in WO 00/61771 A3 on pages 99-100. Transformed plants are grown to maturity and seed that is produced is analyzed for total tocopherol content and composition.

The tocopherol data presented in Tables 3 and 5 demonstrate the reduction of β-tocopherol and more so, δ-tocopherol in favor of γ and α-tocopherol production in soybean seeds harboring a tMT2 expression construct. Tables 4 and 6 demonstrate a nearly complete (98% in the R0 generation) conversion of tocopherols into α-tocopherol in soybean seed harboring a double gene expression construct for tMT2 and a γ-methyltransferase.

Table 6 below depicts the results of the analysis of various soybean lines transformed with pMon67226 Soy. Tables 6 and 9 contain the results of HPLC analysis using the methodology (with minor modifications) described in Savidge et al., *Plant Phys.* 129:321-332 (2000), Isolation and Characterization of Homogentisate Phytltransferase Genes from *Synechocystis* sp PCC 6803 and *Arabidopsis*.

Table 6

Pedigree	% delta	% gamma	% alpha	% beta*		mp:aT	mp:bT	mp:gT	mp:dT	total toco.
A3244	22.90	63.97	10.44	2.69		31	8	190	68	297
A3244	22.85	64.24	10.26	2.65		31	8	194	69	302
A3244	22.88	64.38	10.46	2.29		32	7	197	70	306
A3244	23.08	64.21	10.37	2.34		31	7	192	69	299
A3244	22.97	64.19	10.47	2.36		31	7	190	68	296
GM_A28213:@.	36.92	51.08	8.31	3.69		27	12	166	120	325
GM_A27926:@.	27.51	62.72	7.46	2.31		29	9	244	107	389
GM_A27928:@.	26.56	62.81	8.13	2.50		26	8	201	85	320
GM_A27993:@.	25.70	62.29	9.50	2.51		34	9	223	92	358
GM_A27628:@.	25.07	61.19	10.75	2.99		36	10	205	84	335
GM_A28069:@.	24.66	58.56	13.01	3.77		38	11	171	72	292
GM_A27927:@.	24.41	63.05	10.17	2.37		30	7	186	72	295
GM_A28930:@.	24.14	63.01	10.03	2.82		32	9	201	77	319
GM_A28597:@.	23.89	61.09	11.60	3.41		34	10	179	70	293
GM_A28077:@.	23.73	65.76	8.47	2.03		25	6	194	70	295
GM_A28410:@.	23.70	66.47	7.80	2.02		27	7	230	82	346

Pedigree	% delta	% gamma	% alpha	% beta*	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A28212:@.	23.37	63.91	10.06	2.66	34	9	216	79	338
GM_A28079:@.	23.10	62.38	11.22	3.30	34	10	189	70	303
GM_A27992:@.	23.05	52.42	19.70	4.83	53	13	141	62	269
GM_A28074:@.	22.52	61.86	12.61	3.00	42	10	206	75	333
GM_A28931:@.	20.66	63.28	13.44	2.62	41	8	193	63	305
GM_A28767:@.	20.20	65.66	11.78	2.36	35	7	195	60	297
GM_A28598:@.	20.14	61.09	15.02	3.75	44	11	179	59	293
GM_A28214:@.	20.07	61.90	14.29	3.74	42	11	182	59	294
GM_A28062:@.	19.80	64.09	13.09	3.02	39	9	191	59	298
GM_A28505:@.	19.69	66.77	11.69	1.85	38	6	217	64	325
GM_A28067:@.	18.18	62.55	15.64	3.64	43	10	172	50	275
GM_A28503:@.	18.06	65.63	14.24	2.08	41	6	189	52	288
GM_A28408:@.	17.97	64.75	14.58	2.71	43	8	191	53	295
GM_A28061:@.	17.87	62.20	16.15	3.78	47	11	181	52	291
GM_A28504:@.	17.73	62.06	16.67	3.55	47	10	175	50	282
GM_A28409:@.	16.79	63.14	16.42	3.65	45	10	173	46	274
GM_A28060:@.	16.16	68.35	13.80	1.68	41	5	203	48	297
GM_A28076:@.	16.04	60.41	19.11	4.44	56	13	177	47	293
GM_A28066:@.	15.36	59.73	20.48	4.44	60	13	175	45	293
GM_A29037:@.	14.49	71.59	12.22	1.70	43	6	252	51	352
GM_A27855:@.	13.64	74.68	10.39	1.30	32	4	230	42	308
GM_A27856:@.	13.46	72.76	12.18	1.60	38	5	227	42	312
GM_A28081:@.	11.11	76.85	10.80	1.23	35	4	249	36	324
GM_A27627:@.	8.33	75.93	14.20	1.54	46	5	246	27	324
GM_A27932:@.	8.13	81.33	9.94	0.60	33	2	270	27	332
GM_A27857:@.	7.28	78.48	13.29	0.95	42	3	248	23	316
GM_A28073:@.	7.22	67.70	23.37	1.72	68	5	197	21	291
GM_A27708:@.	7.06	75.77	16.26	0.92	53	3	247	23	326
GM_A28059:@.	6.99	77.57	14.71	0.74	40	2	211	19	272
GM_A27925:@.	6.95	76.82	15.23	0.99	46	3	232	21	302
GM_A27859:@.	6.83	77.34	14.39	1.44	40	4	215	19	278
GM_A28065:@.	6.44	73.22	18.64	1.69	55	5	216	19	295
GM_A27931:@.	6.33	78.92	13.86	0.90	46	3	262	21	332
GM_A28246:@.	6.31	72.24	19.87	1.58	63	5	229	20	317
GM_A27994:@.	6.29	79.02	13.99	0.70	40	2	226	18	286
GM_A27995:@.	6.08	78.12	14.89	0.91	49	3	257	20	329
GM_A28075:@.	5.61	73.60	19.14	1.65	58	5	223	17	303
GM_A28070:@.	5.47	79.42	14.47	0.64	45	2	247	17	311
GM_A28068:@.	4.76	75.85	18.71	0.68	55	2	223	14	294
GM_A28078:@.	3.72	81.08	14.53	0.68	43	2	240	11	296
GM_A28080:@.	3.69	73.06	21.77	1.48	59	4	198	10	271
GM_A28071:@.	3.64	75.83	19.87	0.66	60	2	229	11	302
GM_A28058:@.	3.51	82.16	13.74	0.58	47	2	281	12	342
GM_A28064:@.	2.23	85.03	12.74	0.00	40	0	267	7	314
GM_A28599:@.	1.47	82.65	15.88	0.00	54	0	281	5	340
GM_A27929:@.	1.23	83.74	13.80	1.23	45	4	273	4	326
GM_A28063:@.	1.22	74.62	23.55	0.61	77	2	244	4	327
GM_A28072:@.	0.95	76.66	22.08	0.32	70	1	243	3	317
GM_A27930:@.	0.68	79.05	20.27	0.00	60	0	234	2	296

Table 7 below sets forth the results of the analysis of various soybean lines transformed with pMON 67227.

Table 7

Pedigree	% alpha	% beta*	%gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
A3244	10.4	2.7	64.0	22.9	31	8	190	68	297
A3244	10.3	2.6	64.2	22.8	31	8	194	69	302
A3244	10.5	2.3	64.4	22.9	32	7	197	70	306
A3244	10.4	2.3	64.2	23.1	31	7	192	69	299
A3244	10.5	2.4	64.2	23.0	31	7	190	68	296
GM_A27999:@.	9.5	2.5	62.9	25.2	31	8	205	82	326
GM_A28091:@.	10.5	3.1	61.9	24.5	31	9	182	72	294
GM_A28090:@.	11.3	2.7	63.0	22.9	33	8	184	67	292
GM_A28933:@.	14.4	2.1	65.8	17.7	48	7	219	59	333
GM_A28601:@.	15.7	3.1	62.4	18.8	45	9	179	54	287
GM_A27712:@.	60.4	2.5	26.9	10.2	171	7	76	29	283
GM_A27936:@.	60.6	20.4	13.8	5.2	163	55	37	14	269
GM_A28093:@.	67.2	3.3	21.2	8.3	203	10	64	25	302
GM_A27934:@.	75.4	3.1	16.5	5.0	196	8	43	13	260
GM_A28096:@.	79.1	3.4	12.5	5.0	253	11	40	16	320
GM_A27935:@.	88.5	2.7	6.9	1.9	231	7	18	5	261
GM_A27998:@.	89.6	2.5	6.0	1.9	285	8	19	6	318
GM_A27711:@.	91.4	3.3	4.3	1.0	276	10	13	3	302

Table 8 below sets for the results of the analysis of single seeds of soybean transformed with pMON 67226.

Table 8

Pedigree	% alpha	% beta*	%gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A27930:@.	12.2	3.4	64.1	20.3	29	8	152	48	237
GM_A27930:@.	21.7	0.0	77.9	0.4	55	0	197	1	253
GM_A27930:@.	15.0	0.0	84.0	1.0	46	0	257	3	306
GM_A27930:@.	22.4	0.0	76.8	0.8	58	0	199	2	259
GM_A27930:@.	13.9	0.0	85.7	0.4	33	0	204	1	238
GM_A27930:@.	21.7	0.0	77.6	0.7	63	0	225	2	290
GM_A27930:@.	21.7	0.0	77.6	0.8	55	0	197	2	254
GM_A27930:@.	25.7	0.0	74.0	0.4	68	0	196	1	265
GM_A28072:@.	22.4	0.0	76.8	0.8	57	0	195	2	254
GM_A28072:@.	31.3		67.6	1.2	80	0	173	3	256
GM_A28072:@.	22.8	0.0	76.5	0.7	64	0	215	2	281
GM_A28072:@.	17.6	0.0	81.5	1.0	55	0	255	3	313
GM_A28072:@.	20.0	0.0	78.9	1.1	55	0	217	3	275
GM_A28072:@.	35.0	0.0	64.6	0.4	97	0	179	1	277
GM_A28072:@.	31.5	0.0	68.1	0.4	80	0	173	1	254
GM_A28072:@.	16.4	0.0	82.6	1.0	51	0	257	3	311

Table 9 below sets forth the results of the analysis of single seeds of soybean transformed with pMON 67227.

Table 9

Pedigree	% alpha	% beta*	%gamma	% delta	mp:aT	mp:bT	mp:gT	mp:dT	total toco.
GM_A27711:@.	97.8	2.2	0.0	0.0	263	6	0	0	269
GM_A27711:@.	96.7	3.3	0.0	0.0	320	11	0	0	331
GM_A27711:@.	96.5	3.5	0.0	0.0	301	11	0	0	312
GM_A27711:@.	96.7	3.3	0.0	0.0	295	10	0	0	305
GM_A27711:@.	96.9	3.1	0.0	0.0	308	10	0	0	318
GM_A27711:@.	97.3	2.7	0.0	0.0	287	8	0	0	295
GM_A27711:@.	98.2	1.8	0.0	0.0	272	5	0	0	277
GM_A27711:@.	95.7	4.3	0.0	0.0	287	13	0	0	300
GM_A27935:@.	10.3	2.6	65.4	21.7	28	7	178	59	272
GM_A27935:@.	98.5	1.5	0.0	0.0	261	4	0	0	265
GM_A27935:@.	98.3	1.7	0.0	0.0	230	4	0	0	234
GM_A27935:@.	98.6	1.4	0.0	0.0	272	4	0	0	276
GM_A27935:@.	98.2	1.8	0.0	0.0	267	5	0	0	272
GM_A27935:@.	96.9	3.1	0.0	0.0	277	9	0	0	286
GM_A27935:@.	98.3	1.7	0.0	0.0	337	6	0	0	343
GM_A27935:@.	96.5	3.5	0.0	0.0	276	10	0	0	286
GM_A27998:@.	97.0	3.0	0.0	0.0	318	10	0	0	328
GM_A27998:@.	97.1	2.9	0.0	0.0	300	9	0	0	309
GM_A27998:@.	95.9	4.1	0.0	0.0	324	14	0	0	338
GM_A27998:@.	97.0	3.0	0.0	0.0	292	9	0	0	301
GM_A27998:@.	96.9	3.1	0.0	0.0	314	10	0	0	324
GM_A27998:@.	96.5	3.5	0.0	0.0	359	13	0	0	372
GM_A27998:@.	96.5	3.5	0.0	0.0	335	12	0	0	347
GM_A27998:@.	96.6	3.4	0.0	0.0	310	11	0	0	321
GM_A28096:@.	11.1	3.7	61.0	24.1	36	12	197	78	323
GM_A28096:@.	9.5	3.3	61.4	25.8	29	10	188	79	306
GM_A28096:@.	96.8	3.2	0.0	0.0	299	10	0	0	309
GM_A28096:@.	96.0	4.0	0.0	0.0	288	12	0	0	300
GM_A28096:@.	95.8	4.2	0.0	0.0	319	14	0	0	333
GM_A28096:@.	95.8	4.2	0.0	0.0	295	13	0	0	308
GM_A28096:@.	97.8	2.2	0.0	0.0	316	7	0	0	323
GM_A28096:@.	95.8	4.2	0.0	0.0	300	13	0	0	313

The * next to % beta in Tables 6 through 9 is a label to indicate that β-tocopherol comigrates with an unknown compound, making it difficult to quantify.

***Arabidopsis* double constructs**

The tMT2 gene is cut out of the vector pMON67204 using the restriction enzymes Not I (blunt)/Pst I and then cloned into the napin shuttle vector pCGN3223 which is digested with Sal (blunt)/Pst I. This napin cassette containing the tMT2 gene is then cut out from this vector with *Not* I and the ends are filled in with dNTPs using a Klenow procedure. The resulting fragment is inserted into the vectors pMON16602 (digested with

PmeI) and pCGN10822 (digested with *SnaBI*) to make pMON67224 and pMON67223, respectively (Figures 14 and 15). The vectors pMON16602 and pCGN10822 are described in PCT application WO 0063391.

These double constructs express the tMT2 gene and the prenyltransferase from either *Arabidopsis* (HPT) or *Synechocystis* (*slr*1736) under the control of the napin seed-specific promoter. These constructs are used to transform *Arabidopsis* and transformed plants are grown to maturity, as detailed in Example 6. The resulting T_2 seed is analyzed for total tocopherol content and composition using analytical procedures described in Example 1.

What is claimed is:

1. A substantially purified nucleic acid molecule encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity.

2. The substantially purified nucleic acid molecule of claim 1, wherein said plant is selected from the group consisting of *Arabidopsis thaliana*, Columbia ecotype, *Arabidopsis thaliana*, Landsberg ecotype, corn, soybean, rice, *Allium*, *Brassica*, and *Gossypium*.

3. The substantially purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 through 38.

4. The substantially purified nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a mutant plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity.

5. The substantially purified nucleic acid molecule of claim 4, wherein said nucleic acid molecule is a mutant gene selected from the group consisting of hdt2, hdt6, hdt9, hdt10, and hdt16.

6. A substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 3 through 14, and complements thereof.

7. A substantially purified plant polypeptide molecule having 2-methylphytylplastoquinol methyltransferase activity.

8. The substantially purified plant polypeptide molecule of claim 7, wherein said polypeptide molecule is native to an organism selected from the group consisting of *Arabidopsis thaliana*, Columbia ecotype, *Arabidopsis thaliana*, Landsberg ecotype, corn, soybean, rice, *Allium*, *Brassica*, and *Gossypium*.

9. A transformed plant comprising an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

10. The transformed plant of claim 9, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

11. The transformed plant of claim 9, wherein said transformed plant produces a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

12. The transformed plant of claim 9, wherein said nucleic acid molecule further comprises, in the 5' to 3' direction, a heterologous promoter operably linked to said nucleic acid sequence.

13. The transformed plant of claim 12, wherein said promoter is a seed specific promoter.

14. A transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

15. The transformed plant of claim 14, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

16. The transformed plant of claim 14, wherein said transformed plant produces a seed with one or both of an increased γ-tocopherol level and γ-tocotrienol level relative to

a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

17. The transformed plant of claim 14, wherein said nucleic acid molecule further comprises, in the 5' to 3' direction, a heterologous promoter operably linked to said nucleic acid sequence.

18. The transformed plant of claim 17, wherein said promoter is a seed specific promoter.

19. A transformed plant comprising an introduced nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38.

20. The transformed plant of claim 19, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris, Brassica napus,* oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

21. A transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr 1737, IDI, GGH*, complements thereof, a plant ortholog thereof and an antisense construct for homogentisic acid dioxygenase.

22. The transformed plant of claim 21, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris, Brassica napus,* oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,

grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

23. The transformed plant of claim 21, wherein said introduced second nucleic acid encodes *GMT* and wherein said transformed plant comprises tissue with one or both of an increased α-tocopherol level and increased α-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

24. The transformed plant of claim 21, wherein said transformed plant produces a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

25. The transformed plant of claim 21, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

26. The transformed plant of claim 25, wherein said promoter is a seed specific promoter.

27. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule that encodes an enzyme selected from the group consisting of *tyrA, slr1736, HPT, tocopherol cyclase, dxs, dxr, GGPPS, GMT, HPPD, AANT1, slr 1737, IDI, GGH*, and complements thereof.

28. The transformed plant of claim 27, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

29. The transformed plant of claim 27, wherein said introduced second nucleic acid molecule encodes *GMT* and wherein said transformed plant comprises tissue with one or both of an increased α-tocopherol level and increased α-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

30. The transformed plant of claim 27, wherein said transformed plant produces a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

31. The transformed plant of claim 27, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

32. The transformed plant of claim 31, wherein said promoter is a seed specific promoter.

33. A transformed plant comprising an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

34. The transformed plant of claim 33, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, chick peas, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

35. The transformed plant of claim 33, wherein said transformed plant produces a seed with increased α-tocopherol levels relative to a plant with a similar genetic

background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

36. The transformed plant of claim 33, wherein at least one of said introduced first nucleic acid molecule and said introduced second nucleic acid molecule comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

37. The transformed plant of claim 36, wherein said promoter is a seed specific promoter.

38. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

39. The transformed plant of claim 38, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, citrus, canola, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

40. The transformed plant of claim 38, wherein said transformed plant produces a seed with one or both of an increased α-tocopherol level and increased α-tocotrienol level relative to a plant with a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

41. The transformed plant of claim 38, wherein at least one nucleic acid molecule further comprises, in the 5' to 3' direction, an operably linked heterologous promoter.

42. The transformed plant of claim 41, wherein said promoter is a seed specific promoter.

43. A method for reducing, in a plant, expression of a gene encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity, comprising: (A) transforming a plant with a nucleic acid molecule, said nucleic acid molecule having an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 15, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence; and (B) growing said transformed plant.

44. A transformed plant comprising a nucleic acid molecule comprising an introduced promoter region which functions in plant cells to cause the production of an mRNA molecule, wherein said introduced promoter region is linked to a transcribed nucleic acid molecule having a transcribed strand and a non-transcribed strand, wherein said transcribed strand is complementary to a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1 through 14, and wherein said transcribed nucleic acid molecule is linked to a 3' non-translated sequence that functions in the plant cells to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA sequence.

45. The transformed plant of claim 44, wherein the expression of a gene encoding a plant polypeptide having 2-methylphytylplastoquinol methyltransferase activity is reduced relative to a plant with a similar genetic background but lacking said introduced nucleic acid molecule.

46. A method of producing a plant having a seed with an increased γ-tocopherol level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the

group consisting of SEQ ID NOs: 1, 2, and 8 through 15; and (B) growing said transformed plant.

47. The method of producing a plant of claim 46, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

48. A method of producing a plant having a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, and 33 through 38; and (B) growing said transformed plant.

49. The method of producing a plant of claim 48, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica* napus, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

50. A method of producing a plant having a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr 1737, IDI, GGH*, complements thereof, a plant ortholog,

and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

51. The method of producing a plant of claim 50, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

52. A method of producing a plant having a seed with one or both of an increased γ-tocopherol level and increased γ-tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr 1737, IDI, GGH*, complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase; and (B) growing said transformed plant.

53. The method of producing a plant of claim 52, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

54. A method of producing a plant having a seed with one or both of an increased α-tocopherol level and increased α-tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first

nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and an introduced second nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

55. The method of producing a plant of claim 54, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

56. A method of producing a plant having a seed with one or both of an increased α-tocopherol level and increased α-tocotrienol level comprising: (A) transforming said plant with an introduced first nucleic acid molecule, wherein said first nucleic acid molecule encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, and an introduced second nucleic acid molecule that encodes a polypeptide sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof; and (B) growing said transformed plant.

57. The method of producing a plant of claim 56, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

58. Seed derived from a transformed plant, wherein said transformed plant comprises an introduced nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof.

59. The seed of claim 58, wherein said seed has one or both of an increased γ-tocopherol level and increased γ-tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced nucleic acid molecule.

60. Seed derived from a transformed plant, wherein said transformed plant comprises an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, GMT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr 1737, IDI, GGH*, complements thereof, a plant ortholog thereof and an antisense construct for homogentisic acid dioxygenase.

61. The seed of claim 60, wherein said seed has one or both of an increased γ-tocopherol level and increased γ-tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced first nucleic acid molecule and said second nucleic acid molecule.

62. Seed derived from a transformed plant, wherein said transformed plant comprises an introduced first nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, and an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof.

63. The seed of claim 62, wherein said seed has one or more of an increased α-tocopherol level and increased α-tocotrienol level relative to a seed from a plant having a similar genetic background but lacking said introduced first nucleic acid molecule and said introduced second nucleic acid molecule.

64. A transformed plant comprising an introduced first nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 8 through 15, and complements thereof, an introduced second nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, tocopherol cyclase, dxs, dxr,*

GGPPS, HPPD, AANT1, slr 1737, IDI, GGH, complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase.

65. A transformed plant comprising an introduced first nucleic acid molecule that encodes a polypeptide molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22 through 28, 33 through 38, an introduced second nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NOs: 39 through 54, and complements thereof, and an introduced third nucleic acid molecule encoding an enzyme selected from the group consisting of *tyrA, slr1736, HPT, tocopherol cyclase, dxs, dxr, GGPPS, HPPD, AANT1, slr 1737, IDI, GGH,* complements thereof, a plant ortholog, and an antisense construct for homogentisic acid dioxygenase.

66. A transformed plant comprising an introduced first nucleic acid molecule encoding a tMT2 enzyme and an introduced second nucleic acid molecule encoding a GMT enzyme.

67. A method of producing a plant having seed with an increased α-tocopherol level comprising: (A) transforming said plant with a first nucleic acid molecule encoding a tMT2 enzyme and a second nucleic acid molecule encoding a GMT enzyme; and (B) growing said plant.

68. Oil from the seed of claim 58, 60, or 62.

69. Animal feed comprising the seed of claim 58, 60 or 62.

70. A method of producing a plant having a seed with an increased total tocopherols level comprising: (A) transforming said plant with an introduced nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 8 through 15; and (B) growing said transformed plant.

71. The method of producing a plant of claim 70, wherein said plant is selected from the group consisting of alfalfa, *Arabidopsis thaliana*, barley, *Brassica campestris*, *Brassica napus*, oilseed rape, broccoli, cabbage, canola, citrus, cotton, garlic, oat, *Allium*, flax, an ornamental plant, peanut, pepper, potato, rapeseed, rice, rye, sorghum, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils,

grape, banana, tea, turf grasses, sunflower, soybean, corn, *Phaseolus*, crambe, mustard, castor bean, sesame, cottonseed, linseed, safflower, and oil palm.

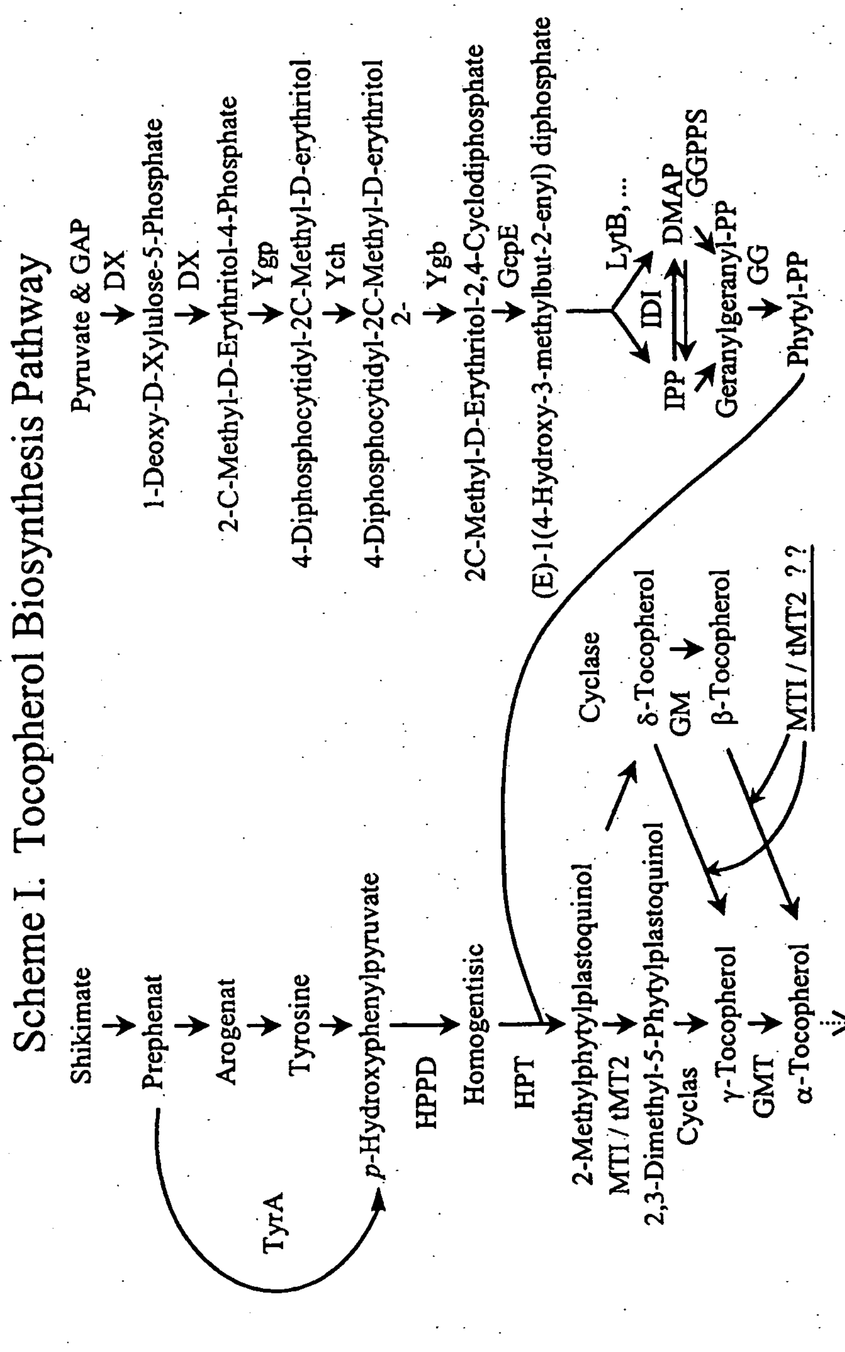
Scheme I. Tocopherol Biosynthesis Pathway
Shikimate
Prephenat
Arogenat
Tyrosine
p-Hydroxyphenylpyruvate
TyrA
HPPD
Homogentisic
HPT
2-Methylphytylplastoquinol
MTI / tMT2
2,3-Dimethyl-5-Phytylplastoquinol
Cyclas
γ-Tocopherol
GMT
α-Tocopherol
Catabolism (?)
Cyclase
δ-Tocopherol
GM
β-Tocopherol
MTI / tMT2 ? ?
Pyruvate & GAP
DX
1-Deoxy-D-Xylulose-5-Phosphate
DX
2-C-Methyl-D-Erythritol-4-Phosphate
Ygp
4-Diphosphocytidyl-2C-Methyl-D-erythritol
Ych
4-Diphosphocytidyl-2C-Methyl-D-erythritol 2-
Ygb
2C-Methyl-D-Erythritol-2,4-Cyclodiphosphate
GcpE
(E)-1(4-Hydroxy-3-methylbut-2-enyl) diphosphate
LytB, ...
IPP
IDI
DMAP
GGPPS
Geranylgeranyl-PP
GG
Phytyl-PP

Fig. 1

```
Query= Arabidopsis TMT2
        (338 letters)

                                                                  Score     E
Sequences producing significant alignments:                       (bits)  Value

Brassica1 - LIB4153-013-R1-K1-B7                                   668     0.0
Brassica2 - LIB80-011-Q1-E1-E9                                     668     0.0
Glycine max TMT2 - LIB3049-032-Q1-E1-G8                            569     e-166
Gossypium hirsutum TMT2 -LIB3272-054-P1-K1-C11                     564     e-164
Allium Porrum - Lib4521-015-Q1-K1-D6                               520     e-151
Zea mays TMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-Q1-K6-G1           508     e-148
Oryza sativa TMT2 - LIB4371-041-R1-K1-F7                           506     e-147

>Brassica1 - LIB4153-013-R1-K1-B7 Length = 337
 Score =  668 bits (1706), Expect = 0.0
 Identities = 322/339 (94%), Positives = 328/339 (95%), Gaps = 3/339 (0%)

>Brassica2 - LIB80-011-Q1-E1-E9 Length = 337
 Score =  668 bits (1706), Expect = 0.0
 Identities = 322/339 (94%), Positives = 328/339 (95%), Gaps = 3/339 (0%)

>Glycine max TMT2 - LIB3049-032-Q1-E1-G8 Length = 342
 Score =  569 bits (1450), Expect = e-166
 Identities = 276/346 (79%), Positives = 298/346 (85%), Gaps = 12/346 (3%)

>Gossypium hirsutum TMT2 -LIB3272-054-P1-K1-C11 Length = 341
 Score =  564 bits (1437), Expect = e-164
 Identities = 274/345 (79%), Positives = 297/345 (85%), Gaps = 11/345 (3%)

>Allium Porrum - Lib4521-015-Q1-K1-D6 Length = 344
 Score =  520 bits (1325), Expect = e-151
 Identities = 261/352 (74%), Positives = 289/352 (81%), Gaps = 22/352 (6%)

>Zea mays TMT2- LIB3587-273-Q1-K6-C5/LIB3600-046-Q1-K6-G1 Length = 352
 Score =  508 bits (1293), Expect = e-148
 Identities = 243/329 (73%), Positives = 276/329 (83%), Gaps = 5/329 (1%)

>Oryza sativa TMT2 - LIB4371-041-R1-K1-F7 Length = 348
 Score =  506 bits (1288), Expect = e-147
 Identities = 247/333 (74%), Positives = 279/333 (83%), Gaps = 7/333 (2%)
```

Fig. 2

```
                                    1                                                   50
SEQ ID NO.  22           corn MAMAST.YAP GGGARA.LAQ GRCRVRGPAG LGFLGPS.KA AGLPRPLALA
SEQ ID NO.  26           rice MAMASSAYAP AGGVGTHSAP G..RIRPPRG LGF...S.TT TTKSRPLVLT
SEQ ID NO.  27      Brassica1 --MASLML.. .NGAITF... .......PKG LGFPASNLHA R..PSPPLSL
SEQ ID NO.  28 ArabidopsisT --MASLML.. .NGAITF... .......PKG LGSPGSNLHA RSIPRPTLLS
SEQ ID NO.  23         Cotton --MASSML.. .NGAETFT.. .LIRGVTPKS IGFLGSGLHG KQFS..SAGL
SEQ ID NO.  25            soy --MGSVML.. .SGTEKLT.. .L.RTLTGNG LGFTGSDLHG KNFPRVSFAA
SEQ ID NO.  24           Leek --MASSML.. .SGAESLS.. .MLRIHHQPK LTFSSPSLHS KPTNLKMDLI
SEQ ID NO. 108      Consensus --M-S----- --G------- ---------- ---------- ----------

                                    51                                                 100
SEQ ID NO.  22           corn LARRMSSPVA VGARLRCAAS SSPAAARPAT APRFIQHKKE AFWFYRFLSI
SEQ ID NO.  26           rice RRGGGGGNIS V.ARLRCAAS SSSAAARPMS QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.  27      Brassica1 VSNTATRRL. .SVATRCSSS SSVSASRPSA QPRFIQHKKE AYWFYRFLSI
SEQ ID NO.  28 ArabidopsisT VTRTSTPRL. .SVATRC.SS SSVSSSRPSA QPRFIQHKKE AYWFYRFLSI
SEQ ID NO.  23         Cotton IYSPKMSRVG TTIAPRC... .SLSASRPAS QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.  25            soy TTSAKVPNFR SIVVPKC... .SVSASRPSS QPRFIQHKKE AFWFYRFLSI
SEQ ID NO.  24           Leek PFATKHQKTK KASIFTCSAS SS...SRPAS QPRFIQHKQE AFWFYRFLSI
SEQ ID NO. 108      Consensus ---------- ------C--- -S----RP-- -PRFIQHK-E A-WFYRFLSI

                                    101                                                150
SEQ ID NO.  22           corn VYDHVINPGH WTEDMRRDDAL EPADLFSRHL TVVDVGGGTG FTTLGIVKHV
SEQ ID NO.  26           rice VYDHVINPGH WTEDMRRDDAL EPADLYSRKL RVVDVGGGTG FTTLGIVKRV
SEQ ID NO.  27      Brassica1 VYDHIINPGH WTEDMRRDDAL EPADLSHPDM RVVDVGGGTG FTTLGIVKTV
SEQ ID NO.  28 ArabidopsisT VYDHVINPGH WTEDMRRDDAL EPADLSHPDM RVVDVGGGTG FTTLGIVKTV
SEQ ID NO.  23         Cotton VYDHVINPGH WTEDMRRDDAL EPADLNDRDM VVVDVGGGTG FTTLGIVQHV
SEQ ID NO.  25            soy VYDHVINPGH WTEDMRRDDAL EPADLNDRNM IVVDVGGGTG FTTLGIVKHV
SEQ ID NO.  24           Leek VYDHVINPGH WTEDMRRDDAL EPAELYDSRM KVVDVGGGTG FTTLGIIKHI
SEQ ID NO. 108      Consensus VYDH-INPGH WTEDMRRDDAL EPA-L----- -VVDVGGGTG FTTLGI----

                                    151                                                200
SEQ ID NO.  22           corn NPENVTLLDQ SPHQLDKARQ KEALKGVTIM EGDAEDLPFP TDSFDRYISA
SEQ ID NO.  26           rice DPENVTLLDQ SPHQLEKARE KEALKGVTIM EGDAEDLPFP TDTFDRYVSA
SEQ ID NO.  27      Brassica1 KAKNVTILDQ SPHQLAKAKQ KEPLKECKIV EGDAEDLPFP TDYADRYVSA
SEQ ID NO.  28 ArabidopsisT KAKNVTILDQ SPHQLAKAKQ KEPLKECKIV EGDAEDLPFP TDYADRYVSA
SEQ ID NO.  23         Cotton DAKNVTILDQ SPHQLAKAKQ KEPLKECNII EGDAEDLPFP TDYADRYVSA
SEQ ID NO.  25            soy DAKNVTILDQ SPHQLAKAKQ KEPLKECKII EGDAEDLPFR TDYADRYVSA
SEQ ID NO.  24           Leek DPKNVTILDQ SPHQLEKARQ KEALKECTIV EGDAEDLPFP TDTFDRYVSA
SEQ ID NO. 108      Consensus ---NVT-LDQ SPHQL-KA-- KE-LK---I- EGDAEDLPF- TD--DRY-SA
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Fig. 3a

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                                        201                                                 250
SEQ ID NO.  22            corn GSIEYWPDPQ RGIKEAYRVL RFGGLACVIG PVYPTFWLSR FFADMWMLFP
SEQ ID NO.  26            rice GSIEYWPDPQ RGIKEAYRVL RLGGVACMIG PVHPTFWLSR FFADMWMLFP
SEQ ID NO.  27       Brassical GSIEYWPDPQ RGIREAYRVL KIGGKACLIG PVHPTFWLSR FFADVWMLFP
SEQ ID NO.  28 ArabidopsisT GSIEYWPDPQ RGIREAYRVL KIGGKACLIG PVYPTFWLSR FFSDVWMLFP
SEQ ID NO.  23          Cotton GSIEYWPDPQ RGIKEAYRVL KQGGKACLIG PVYPTFWLSR FFADVWMLFP
SEQ ID NO.  25             soy GSIEYWPDPQ RGIKEAYRVL KLGGKACLIG PVYPTFWLSR FFADVWMLFP
SEQ ID NO.  24            Leek GSIEYWPDPQ RGIKEAYRVL KLGGVACLIG PVHPTFWLSR FFADMWMLFP
SEQ ID NO. 108       Consensus GSIEYWPDPQ RGI-EAYRVL --GG-AC-IG PV-PTFWLSR FF-D-WMLFP

                                251                                                 300
SEQ ID NO.  22            corn KEEEYIEWFK KAGFRDVKLK RIGPKWYRGV RRHGLIMGCS VTGVKRERGD
SEQ ID NO.  26            rice KEEEYIEWFK KAGFKDVKLK RIGPKWYRGV RRHGLIMGCS VTGVKREHGD
SEQ ID NO.  27       Brassical KEEEYIEWFK NAGFKDVQLK RIGPKWYRGV RRHGLIMGCS VTGVKPASGD
SEQ ID NO.  28 ArabidopsisT KEEEYIEWFK NAGFKDVQLK RIGPKWYRGV RRHGLIMGCS VTGVKPASGD
SEQ ID NO.  23          Cotton KEEEYIEWFE KAGFKDVQLK RIGPKWYRGV RRHGLIMGCS VTGVKPASGD
SEQ ID NO.  25             soy KEEEYIEWFQ KAGFKDVQLK RIGPKWYRGV RRHGLIMGCS VTGVKPASGD
SEQ ID NO.  24            Leek TEEEYIEWFK KAGFKDVKLK RIGPKWYRGV RRHGLIMGCS VTGVKRLSGD
SEQ ID NO. 108       Consensus -EEEYIEWF- -AGF-DV-LK RIGPKWYRGV RRHGLIMGCS VTGVK---GD

                                301                                                 350
SEQ ID NO.  22            corn SPLELGPKAE DVSKPV.NPI TFLFRFLVGT ICAAYYVLVP IYMWIKDQIV
SEQ ID NO.  26            rice SPLQLGPKVE DVSKPV.NPI TFLFRFLMGT ICAAYYVLVP IYMWIKDQIV
SEQ ID NO.  27       Brassical SPLQLGPKEE DVEKPVNNPF SFLGRFLLGT LAAAWFVLIP IYMWIKDQIV
SEQ ID NO.  28 ArabidopsisT SPLQLGPKEE DVEKPVNNPF SFLGRFLLGT LAAAWFVLIP IYMWIKDQIV
SEQ ID NO.  23          Cotton SPLQLGPKAE DVSKPV.NPF VFLLRFMLGA TAAAYYVLVP IYMWLKDQIV
SEQ ID NO.  25             soy SPLQLGPKEE DVEKPV.NPF VFALRFVLGA LAATWFVLVP IYMWLKDQVV
SEQ ID NO.  24            Leek SPLQLGPKAE DVKKPI.NPF SFLLRFILGT IAATYYVLVP IYMWIKDQIV
SEQ ID NO. 108       Consensus SPL-LGPK-E DV-KP--NP- -F--RF--G- --A---VL-P IYMW-KDQ-V

                                351
SEQ ID NO.  22            corn PKGMPI
SEQ ID NO.  26            rice PKGMPI
SEQ ID NO.  27       Brassical PKDQPI
SEQ ID NO.  28 ArabidopsisT PKDQPI
SEQ ID NO.  23          Cotton PEGQPI
SEQ ID NO.  25             soy PKGQPI
SEQ ID NO.  24            Leek PKGQPI
SEQ ID NO. 108       Consensus P---PI
```

Fig. 3b

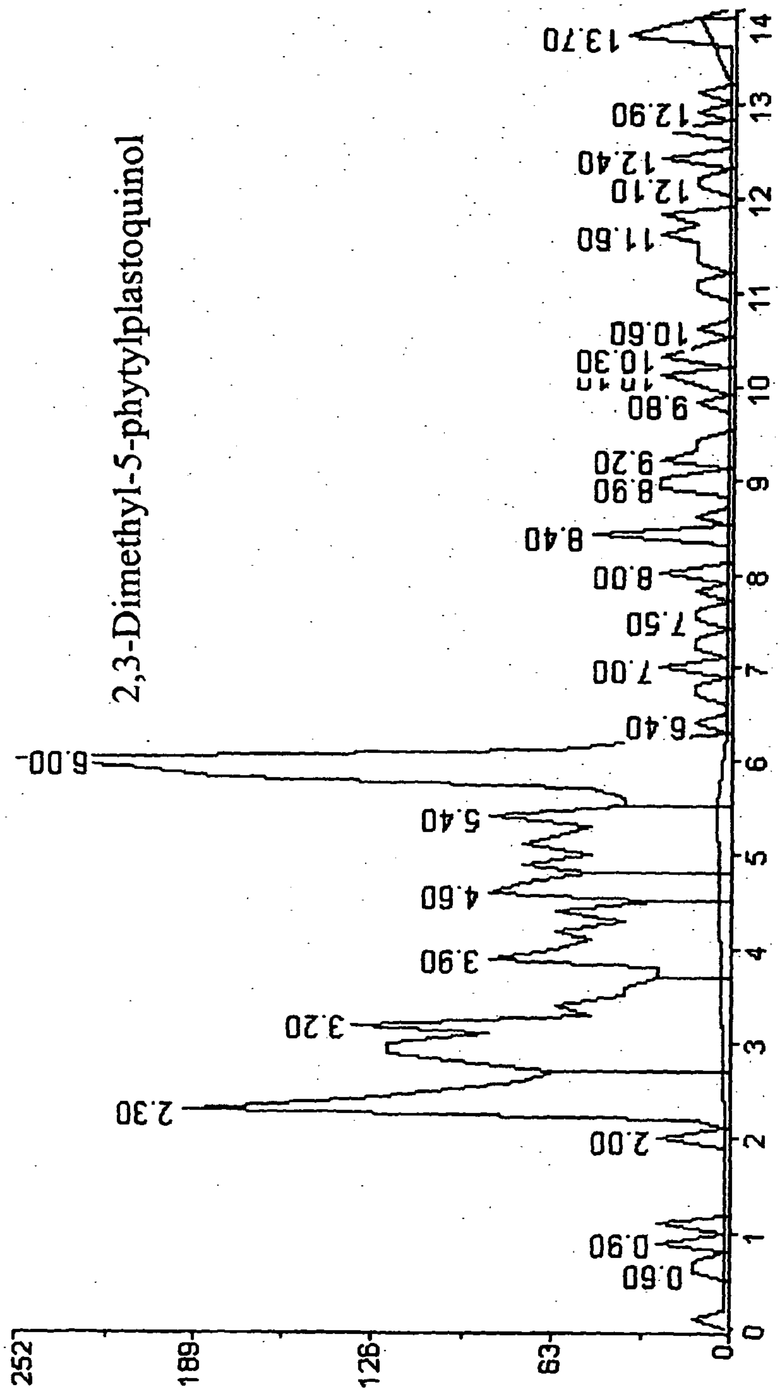

2,3-Dimethyl-5-phytylplastoquinol
0.60
0.90
2.00
2.30
3.20
3.90
4.60
5.40
6.00
6.40
7.00
7.50
8.00
8.40
8.90
9.20
9.80
10.30
10.60
11.60
12.10
12.40
12.90
13.70
0
63
126
189
252
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 4

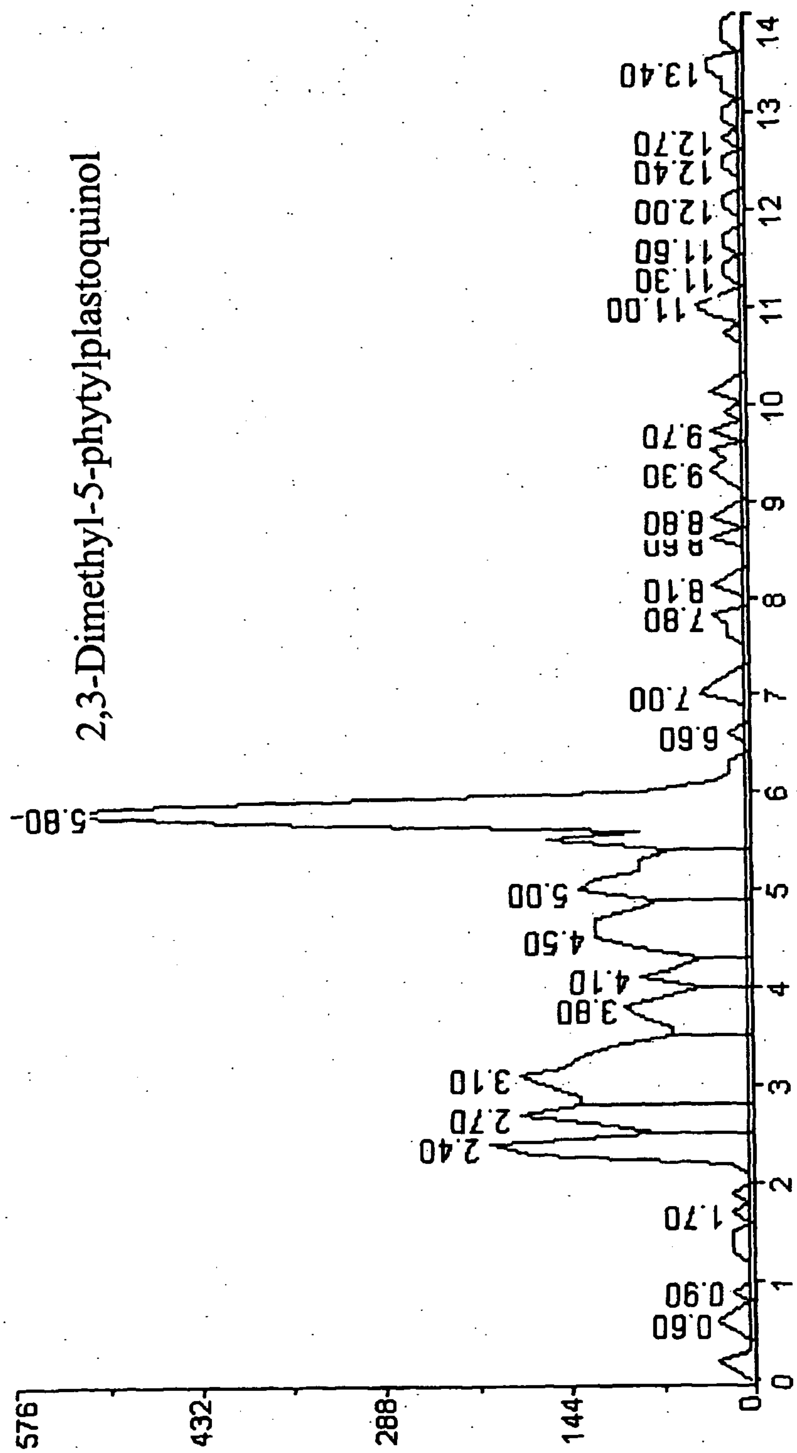

2,3-Dimethyl-5-phytylplastoquinol
576
432
288
144
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
0.60
0.90
1.70
2.40
2.70
3.10
3.80
4.10
4.50
5.00
5.80
6.60
7.00
7.80
8.10
8.50
8.80
9.30
9.70
11.00
11.30
11.60
12.00
12.40
12.70
13.40

Fig. 5

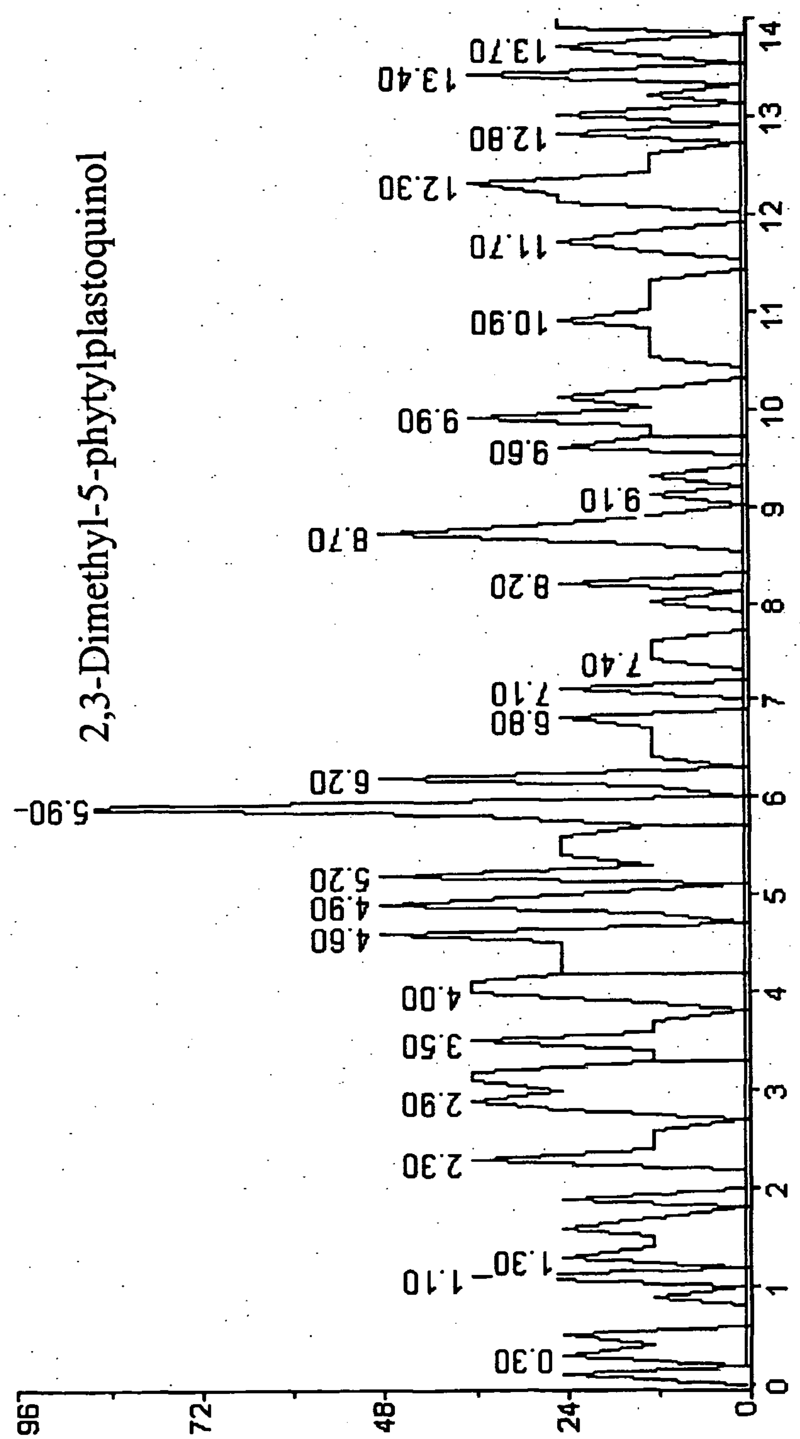

2,3-Dimethyl-5-phytylplastoquinol
0.30
1.10
1.30
2.30
2.90
3.50
4.00
4.60
4.90
5.20
5.90
6.20
6.80
7.10
7.40
8.20
8.70
9.10
9.60
9.90
10.90
11.70
12.30
12.80
13.40
13.70
0
24
48
72
96
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 6

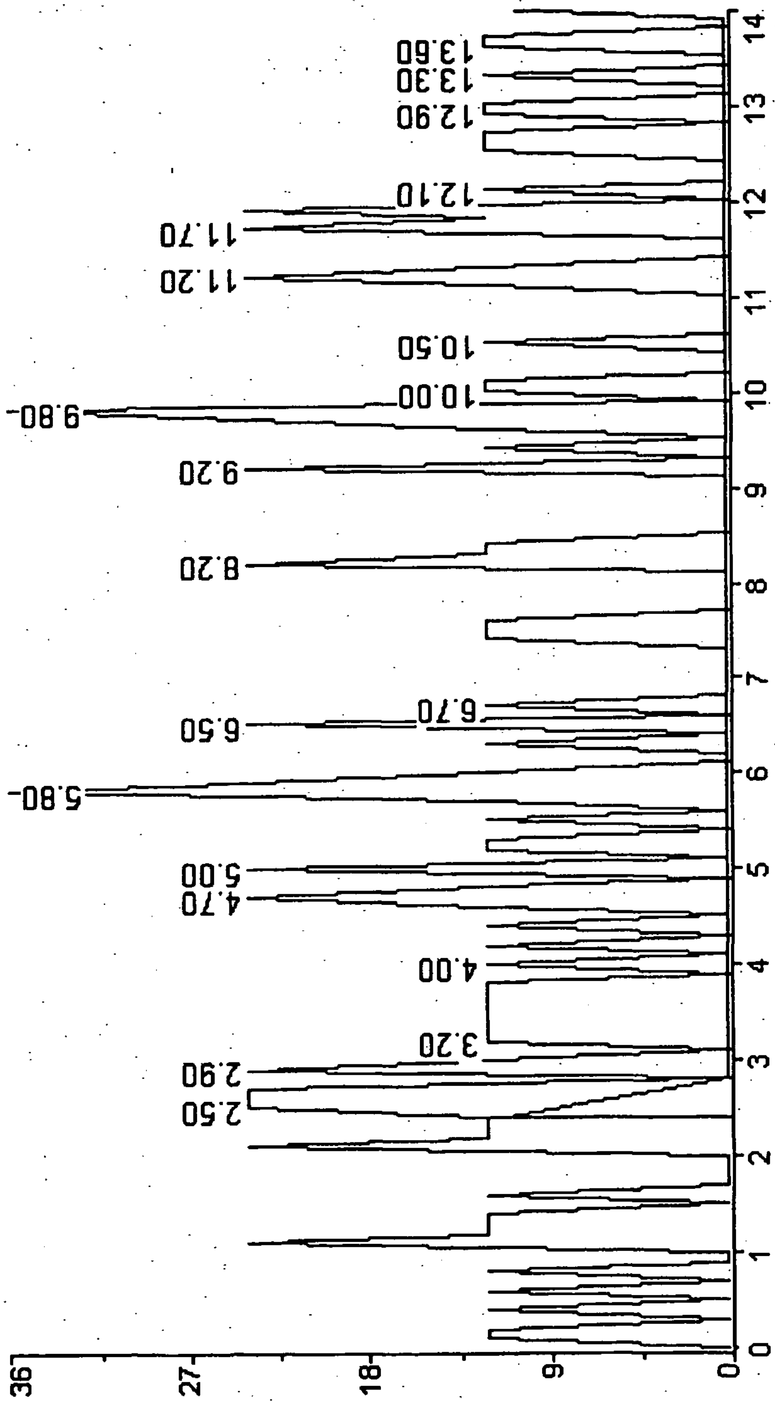

2.50
2.90
3.20
4.00
4.70
5.00
5.80
6.50
6.70
8.20
9.20
9.80
10.00
10.50
11.20
11.70
12.10
12.90
13.30
13.60
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
9
18
27
36

Fig. 7

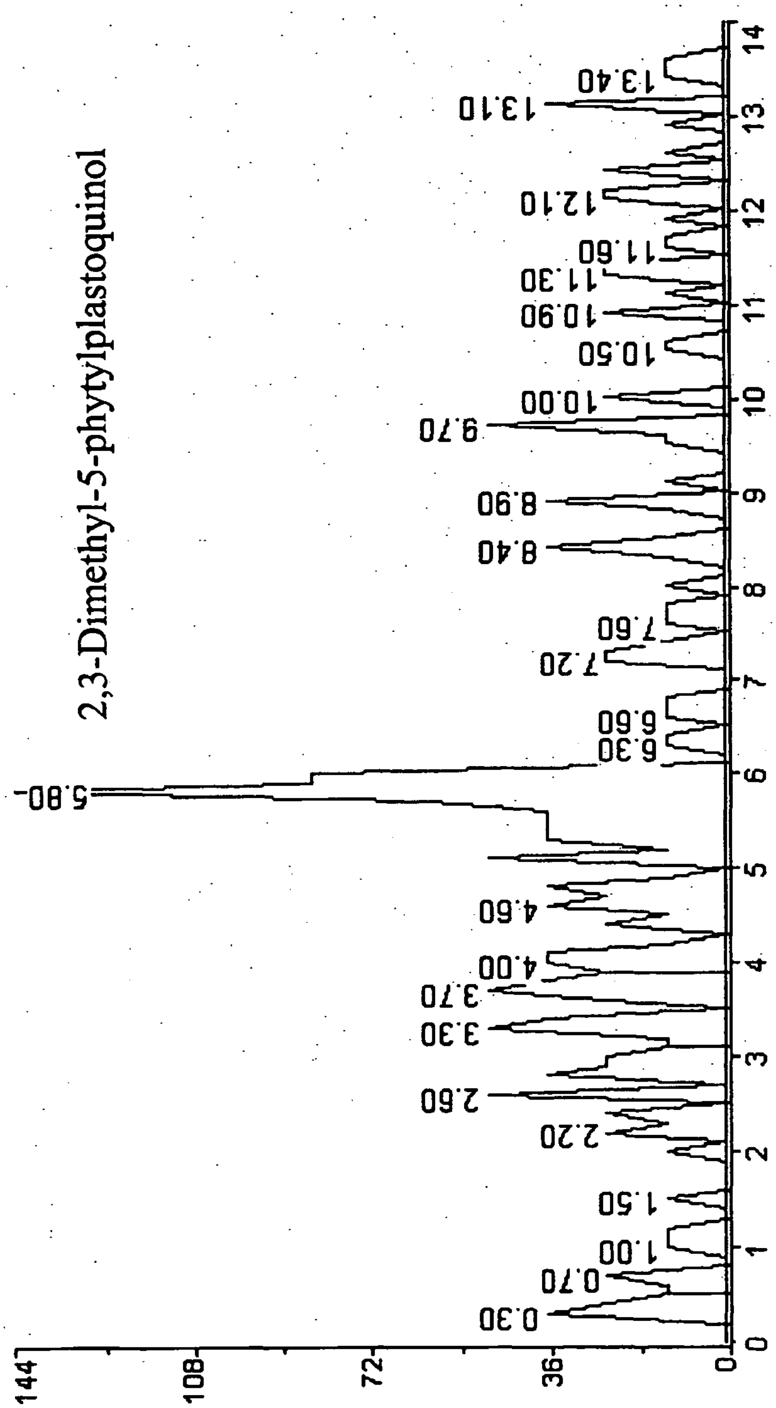

2,3-Dimethyl-5-phytylplastoquinol
0.30
0.70
1.00
1.50
2.20
2.60
3.30
3.70
4.00
4.60
5.80
6.30
6.60
7.20
7.60
8.40
8.90
9.70
10.00
10.50
10.90
11.30
11.60
12.10
13.10
13.40
144
108
72
36
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 8

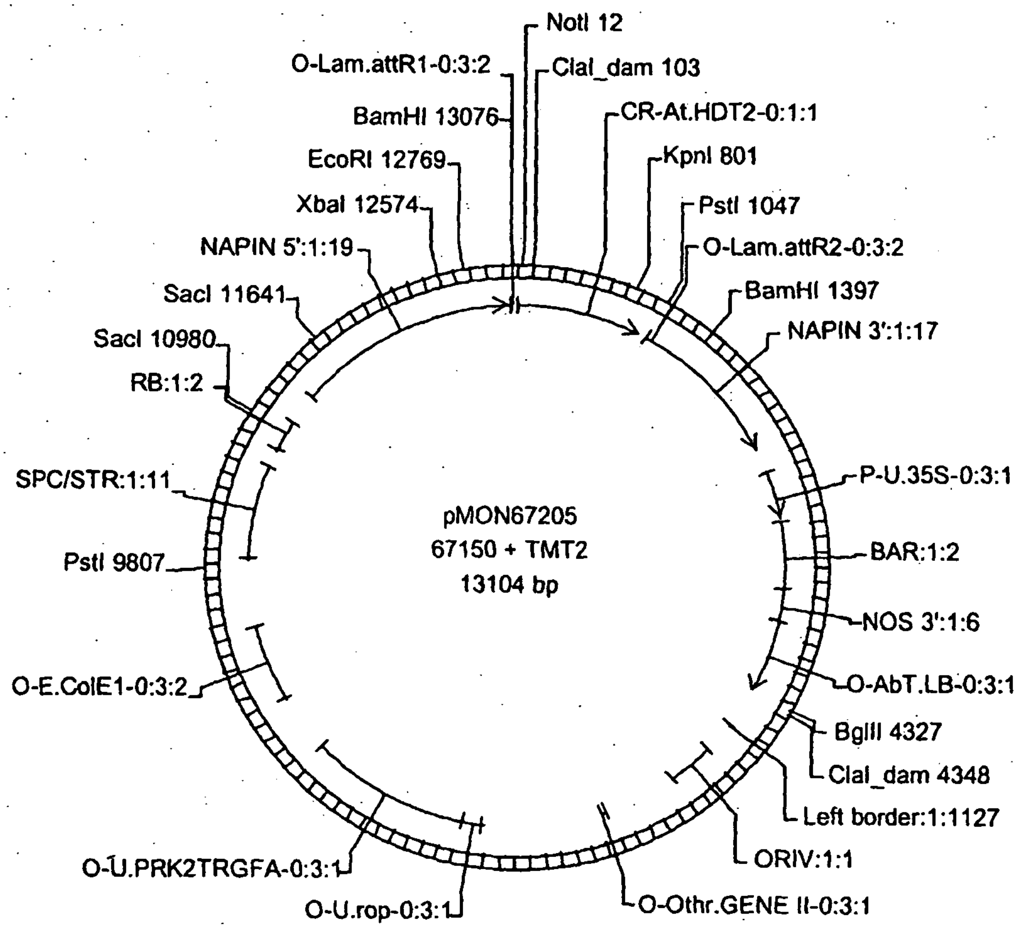

NotI 12
O-Lam.attR1-0:3:2
ClaI_dam 103
BamHI 13076
CR-At.HDT2-0:1:1
EcoRI 12769
KpnI 801
XbaI 12574
PstI 1047
NAPIN 5':1:19
O-Lam.attR2-0:3:2
SacI 11641
BamHI 1397
SacI 10980
NAPIN 3':1:17
RB:1:2
SPC/STR:1:11
P-U.35S-0:3:1
pMON67205
67150 + TMT2
13104 bp
PstI 9807
BAR:1:2
NOS 3':1:6
O-E.ColE1-0:3:2
O-AbT.LB-0:3:1
BglII 4327
ClaI_dam 4348
Left border:1:1127
ORIV:1:1
O-U.PRK2TRGFA-0:3:1
O-U.rop-0:3:1
O-Othr.GENE II-0:3:1

Fig. 9

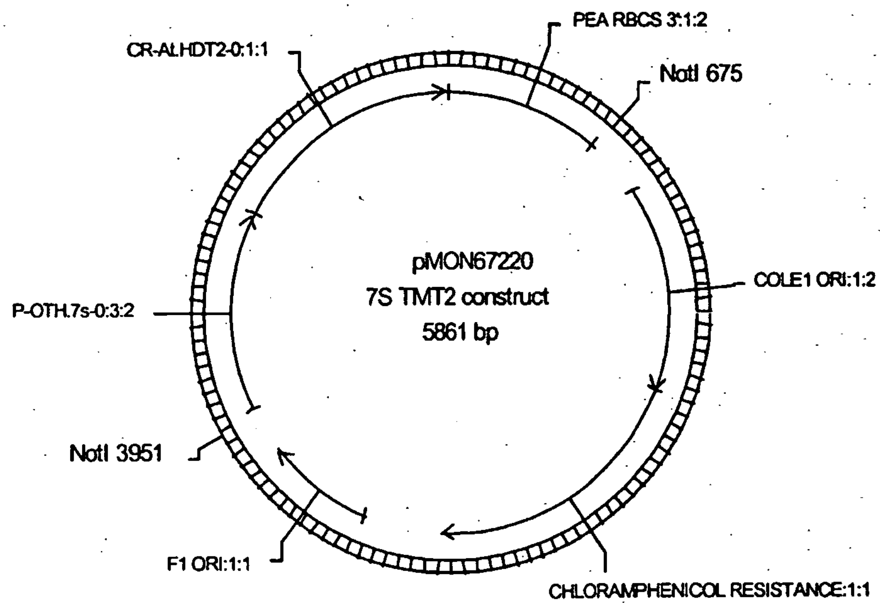

PEA RBCS 3:1:2
CR-ALHDT2-0:1:1
NotI 675
pMON67220
7S TMT2 construct
5861 bp
COLE1 ORI:1:2
P-OTH.7s-0:3:2
NotI 3951
F1 ORI:1:1
CHLORAMPHENICOL RESISTANCE:1:1

Fig. 10

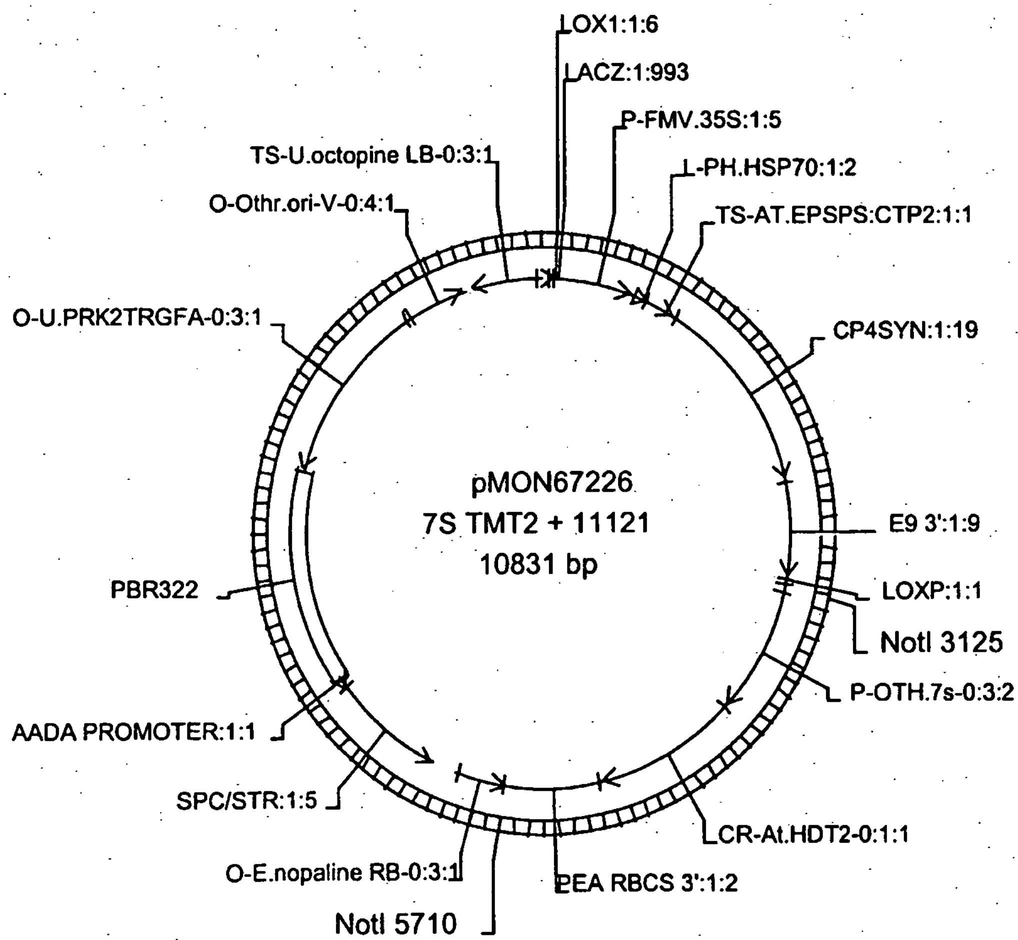

LOX1:1:6
LACZ:1:993
P-FMV.35S:1:5
L-PH.HSP70:1:2
TS-AT.EPSPS:CTP2:1:1
TS-U.octopine LB-0:3:1
O-Othr.ori-V-0:4:1
O-U.PRK2TRGFA-0:3:1
CP4SYN:1:19
pMON67226
7S TMT2 + 11121
10831 bp
E9 3':1:9
PBR322
LOXP:1:1
NotI 3125
P-OTH.7s-0:3:2
AADA PROMOTER:1:1
SPC/STR:1:5
CR-At.HDT2-0:1:1
O-E.nopaline RB-0:3:1
PEA RBCS 3':1:2
NotI 5710

Fig. 11

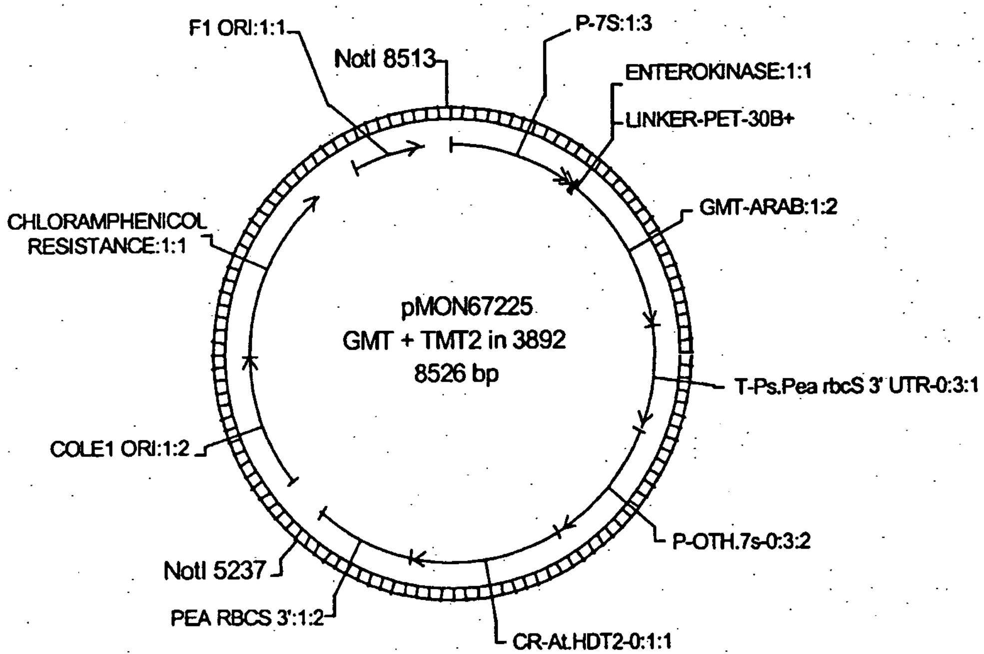

F1 ORI:1:1
NotI 8513
P-7S:1:3
ENTEROKINASE:1:1
LINKER-PET-30B+
GMT-ARAB:1:2
CHLORAMPHENICOL RESISTANCE:1:1
pMON67225
GMT + TMT2 in 3892
8526 bp
T-Ps.Pea rbcS 3' UTR-0:3:1
COLE1 ORI:1:2
P-OTH.7s-0:3:2
NotI 5237
PEA RBCS 3':1:2
CR-At.HDT2-0:1:1

Fig. 12

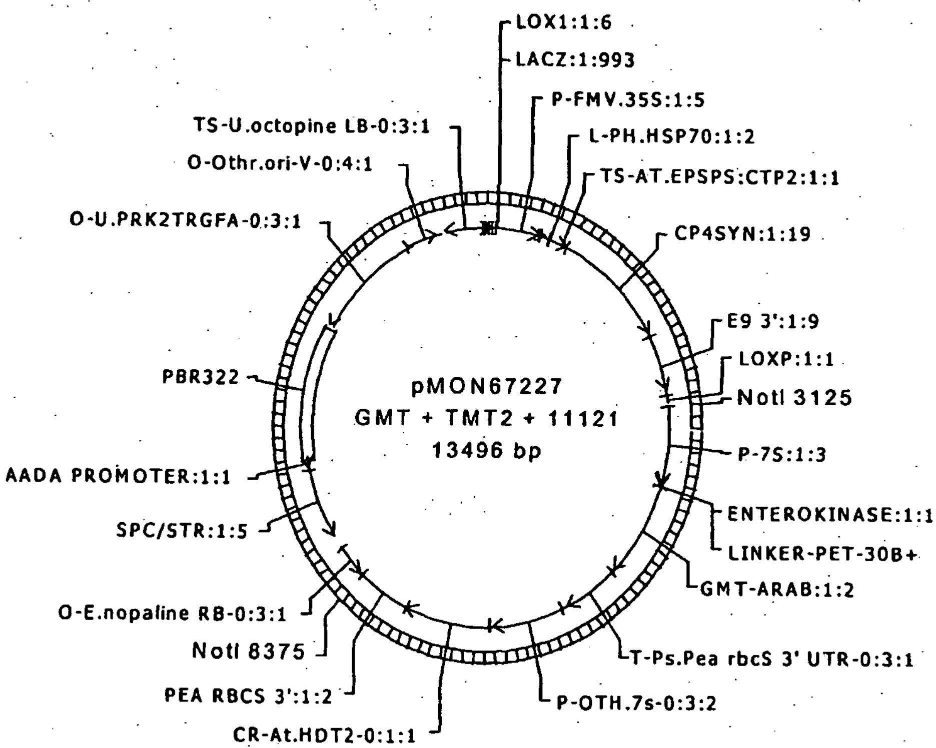

LOX1:1:6
LACZ:1:993
P-FMV.35S:1:5
L-PH.HSP70:1:2
TS-AT.EPSPS:CTP2:1:1
CP4SYN:1:19
E9 3':1:9
LOXP:1:1
NotI 3125
P-7S:1:3
ENTEROKINASE:1:1
LINKER-PET-30B+
GMT-ARAB:1:2
T-Ps.Pea rbcS 3' UTR-0:3:1
P-OTH.7s-0:3:2
CR-At.HDT2-0:1:1
PEA RBCS 3':1:2
NotI 8375
O-E.nopaline RB-0:3:1
SPC/STR:1:5
AADA PROMOTER:1:1
PBR322
O-U.PRK2TRGFA-0:3:1
O-Othr.ori-V-0:4:1
TS-U.octopine LB-0:3:1
pMON67227
GMT + TMT2 + 11121
13496 bp

Fig. 13

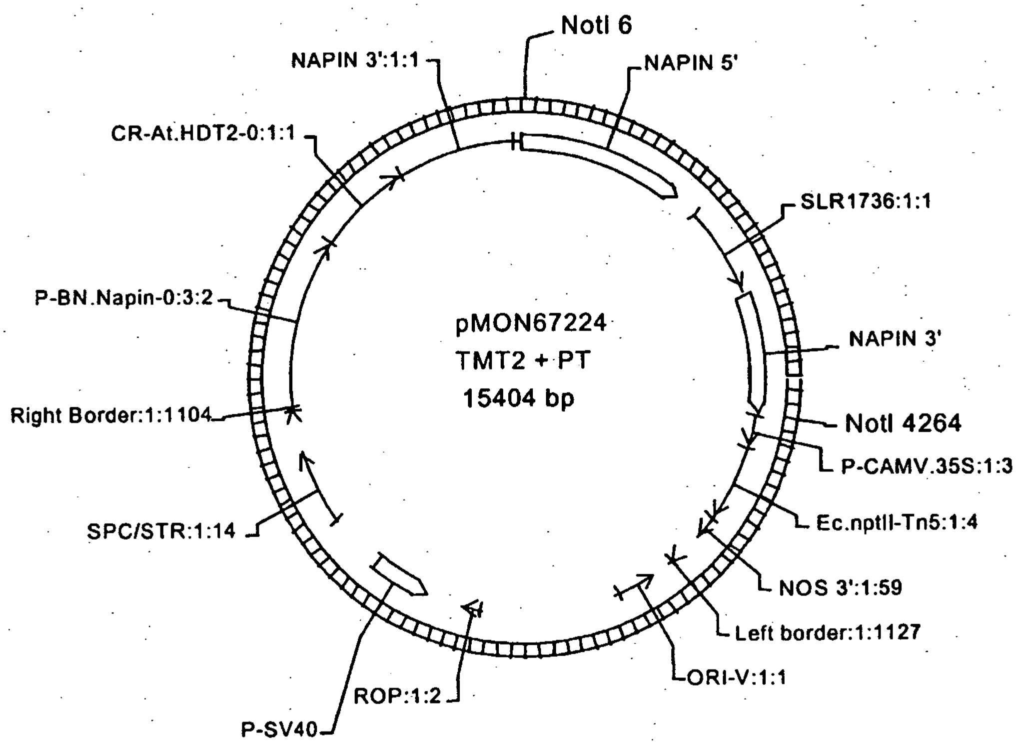

NotI 6
NAPIN 3':1:1
NAPIN 5'
CR-At.HDT2-0:1:1
SLR1736:1:1
P-BN.Napin-0:3:2
pMON67224
TMT2 + PT
15404 bp
NAPIN 3'
NotI 4264
Right Border:1:1104
P-CAMV.35S:1:3
Ec.nptII-Tn5:1:4
SPC/STR:1:14
NOS 3':1:59
Left border:1:1127
ORI-V:1:1
ROP:1:2
P-SV40

Fig. 14

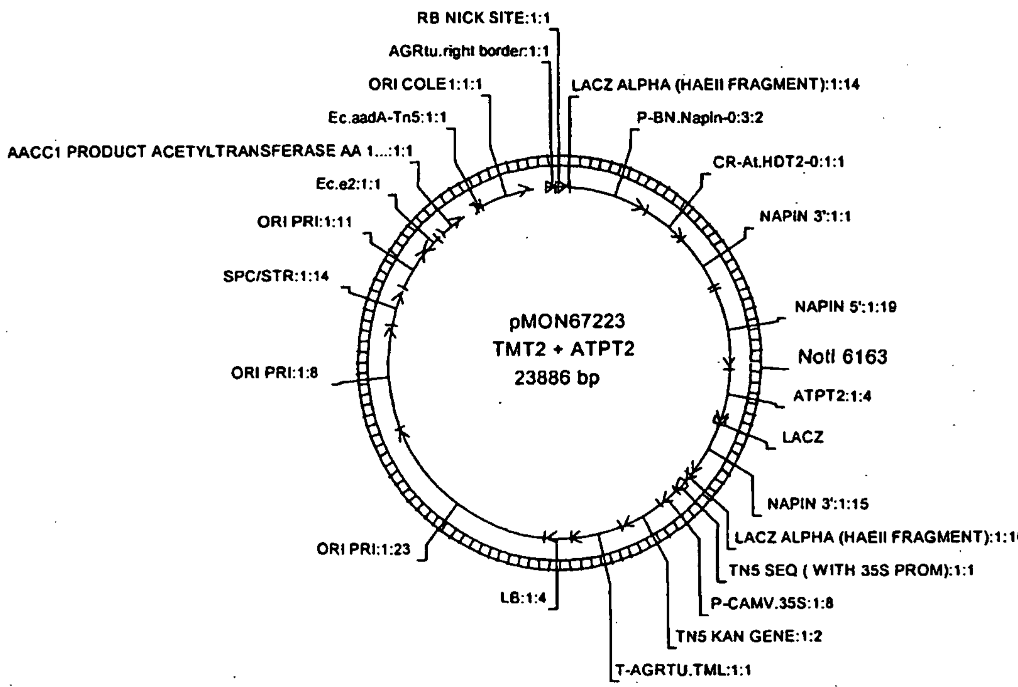
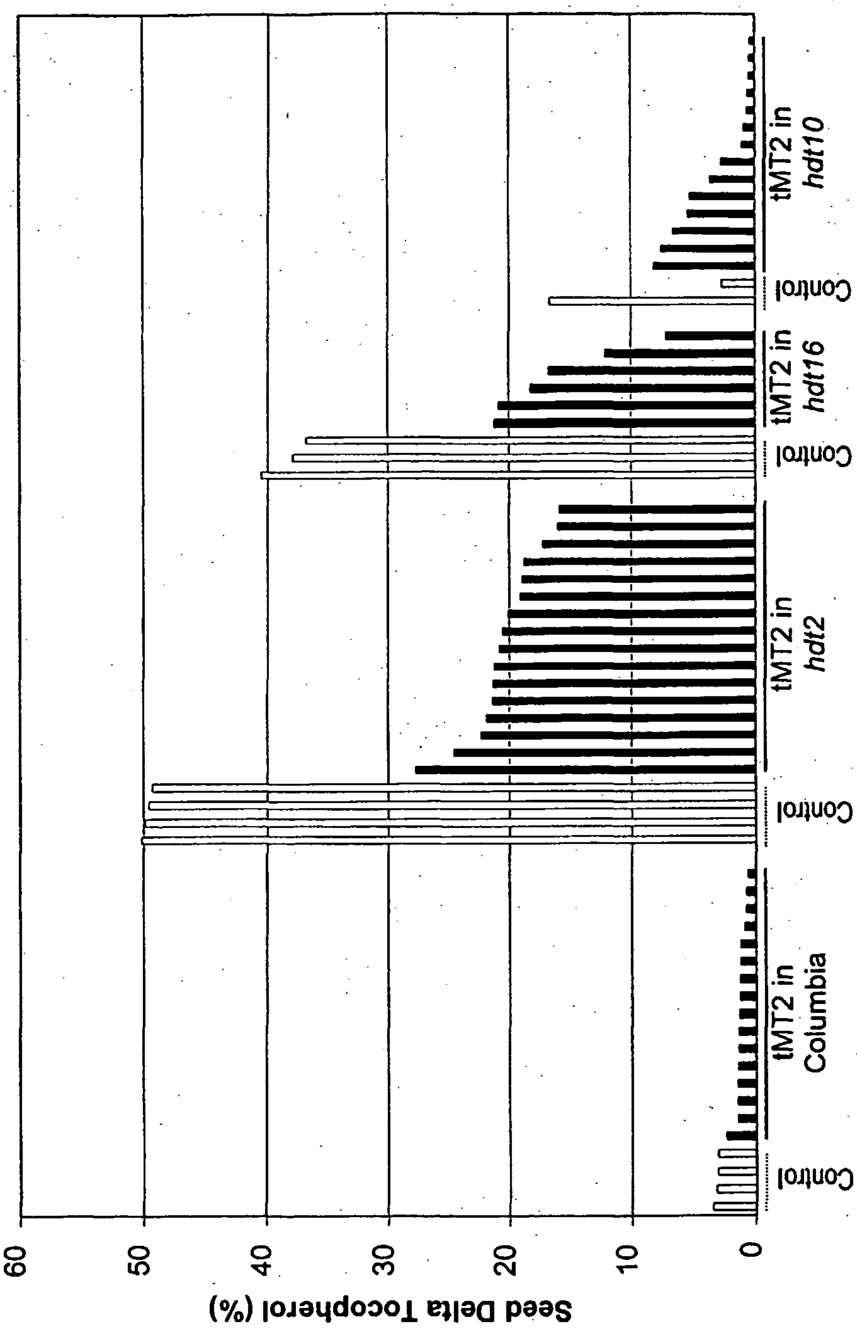
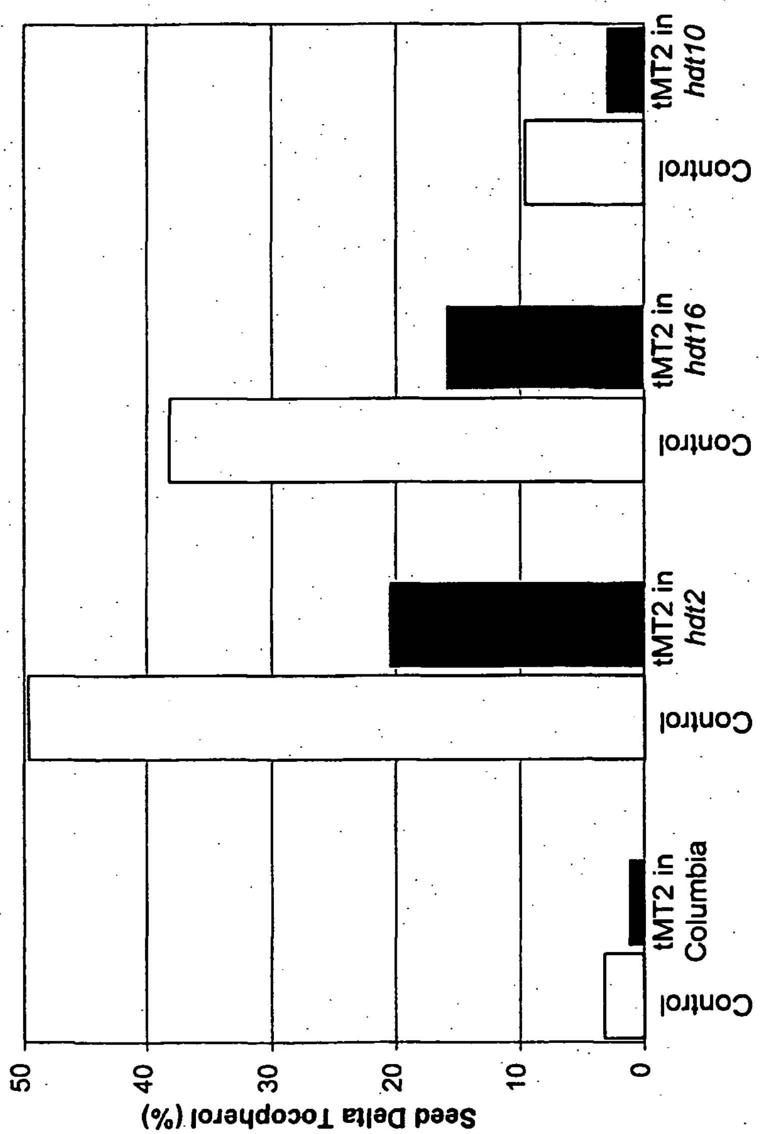

RB NICK SITE:1:1
AGRtu.right border:1:1
ORI COLE1:1:1
Ec.aadA-Tn5:1:1
AACC1 PRODUCT ACETYLTRANSFERASE AA 1...:1:1
Ec.e2:1:1
ORI PRI:1:11
SPC/STR:1:14
ORI PRI:1:8
ORI PRI:1:23
LB:1:4
T-AGRTU.TML:1:1
TN5 KAN GENE:1:2
P-CAMV.35S:1:8
TN5 SEQ (WITH 35S PROM):1:1
LACZ ALPHA (HAEII FRAGMENT):1:16
NAPIN 3':1:15
LACZ
ATPT2:1:4
NotI 6163
NAPIN 5':1:19
NAPIN 3':1:1
CR-At.HDT2-0:1:1
P-BN.Napin-0:3:2
LACZ ALPHA (HAEII FRAGMENT):1:14
pMON67223
TMT2 + ATPT2
23886 bp

Fig. 15



Arabidopsis

Fig. 16a



Arabidopsis

Fig. 16b

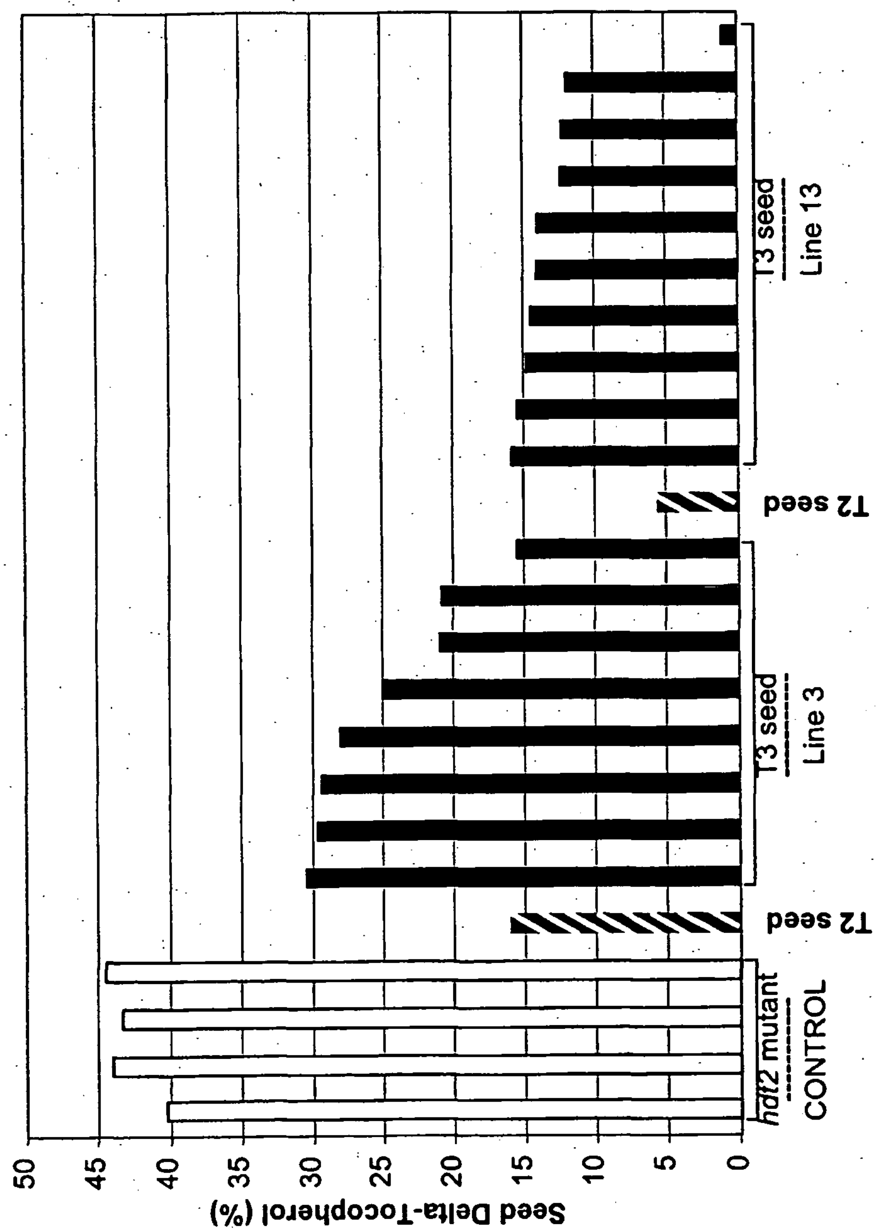

Seed Delta-Tocopherol (%)
0
5
10
15
20
25
30
35
40
45
50
hdt2 mutant
CONTROL
T2 seed
T3 seed
Line 3
T2 seed
T3 seed
Line 13

Fig. 17

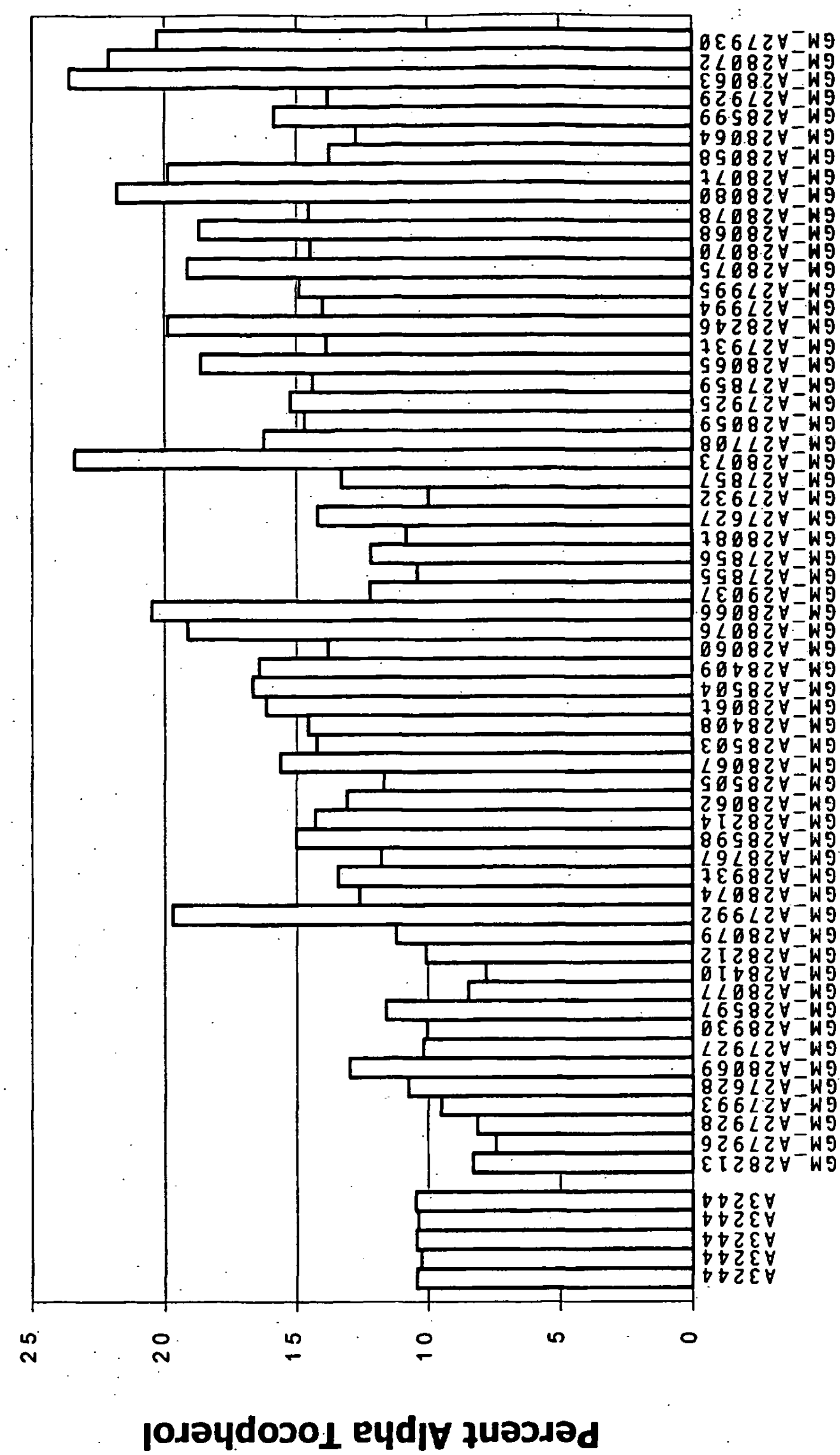
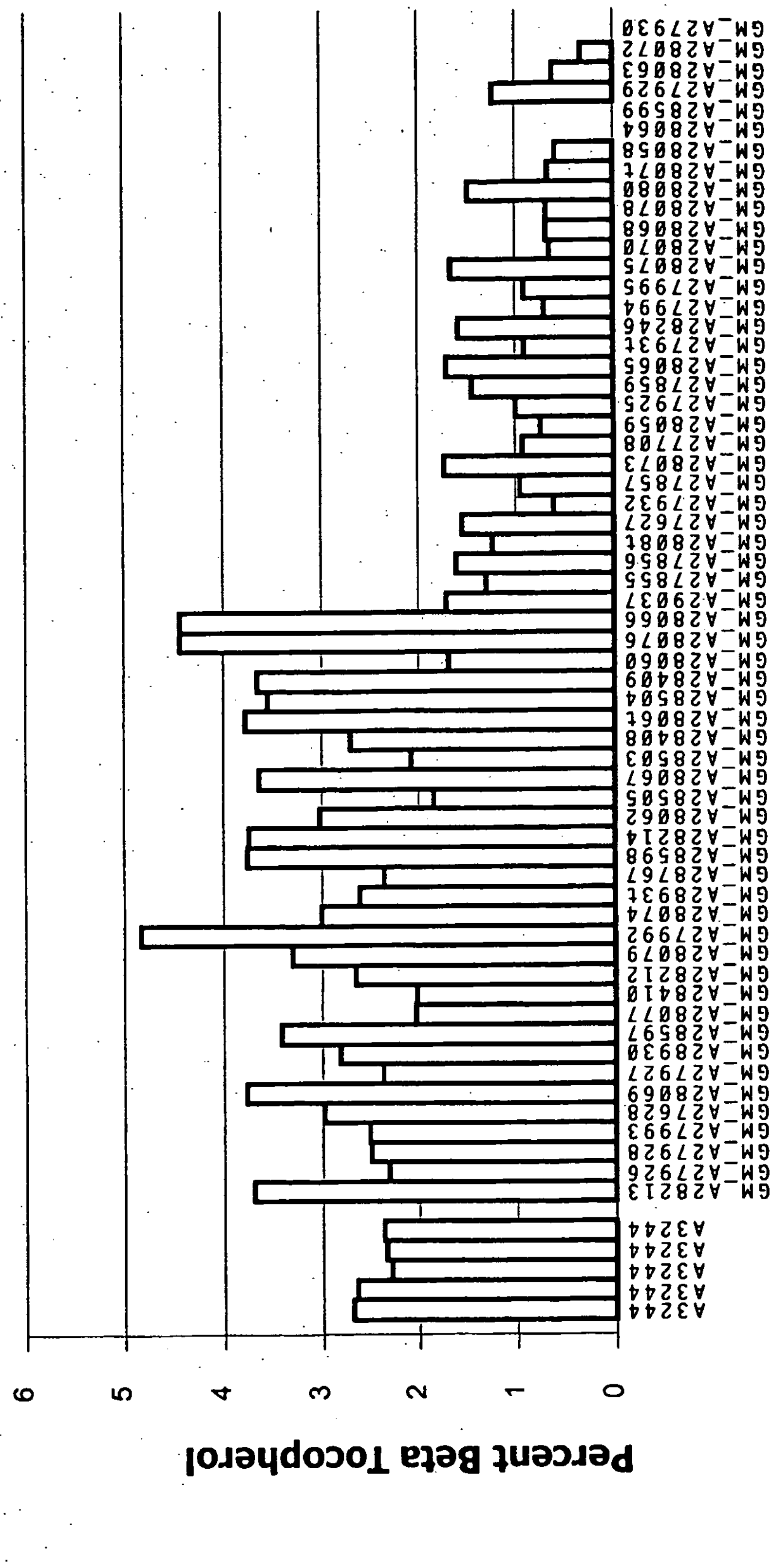

Percent Alpha Tocopherol
0
5
10
15
20
25
A3244
A3244
A3244
A3244
A3244
GM_A28213
GM_A27926
GM_A27928
GM_A27993
GM_A27628
GM_A28069
GM_A27927
GM_A28930
GM_A28597
GM_A28077
GM_A28410
GM_A28212
GM_A28079
GM_A27992
GM_A28074
GM_A28931
GM_A28767
GM_A28598
GM_A28214
GM_A28062
GM_A28505
GM_A28067
GM_A28503
GM_A28408
GM_A28061
GM_A28504
GM_A28409
GM_A28060
GM_A28076
GM_A28066
GM_A29037
GM_A27855
GM_A27856
GM_A28081
GM_A27627
GM_A27932
GM_A27857
GM_A28073
GM_A27708
GM_A28059
GM_A27925
GM_A27859
GM_A28065
GM_A27931
GM_A28246
GM_A27994
GM_A27995
GM_A28075
GM_A28070
GM_A28068
GM_A28078
GM_A28080
GM_A28071
GM_A28058
GM_A28064
GM_A28599
GM_A27929
GM_A28063
GM_A28072
GM_A27930

Fig. 18a


Percent Beta Tocopherol
0
1
2
3
4
5
6
GM_A27930
GM_A28072
GM_A28063
GM_A27929
GM_A28599
GM_A28064
GM_A28058
GM_A28071
GM_A28080
GM_A28078
GM_A28068
GM_A28070
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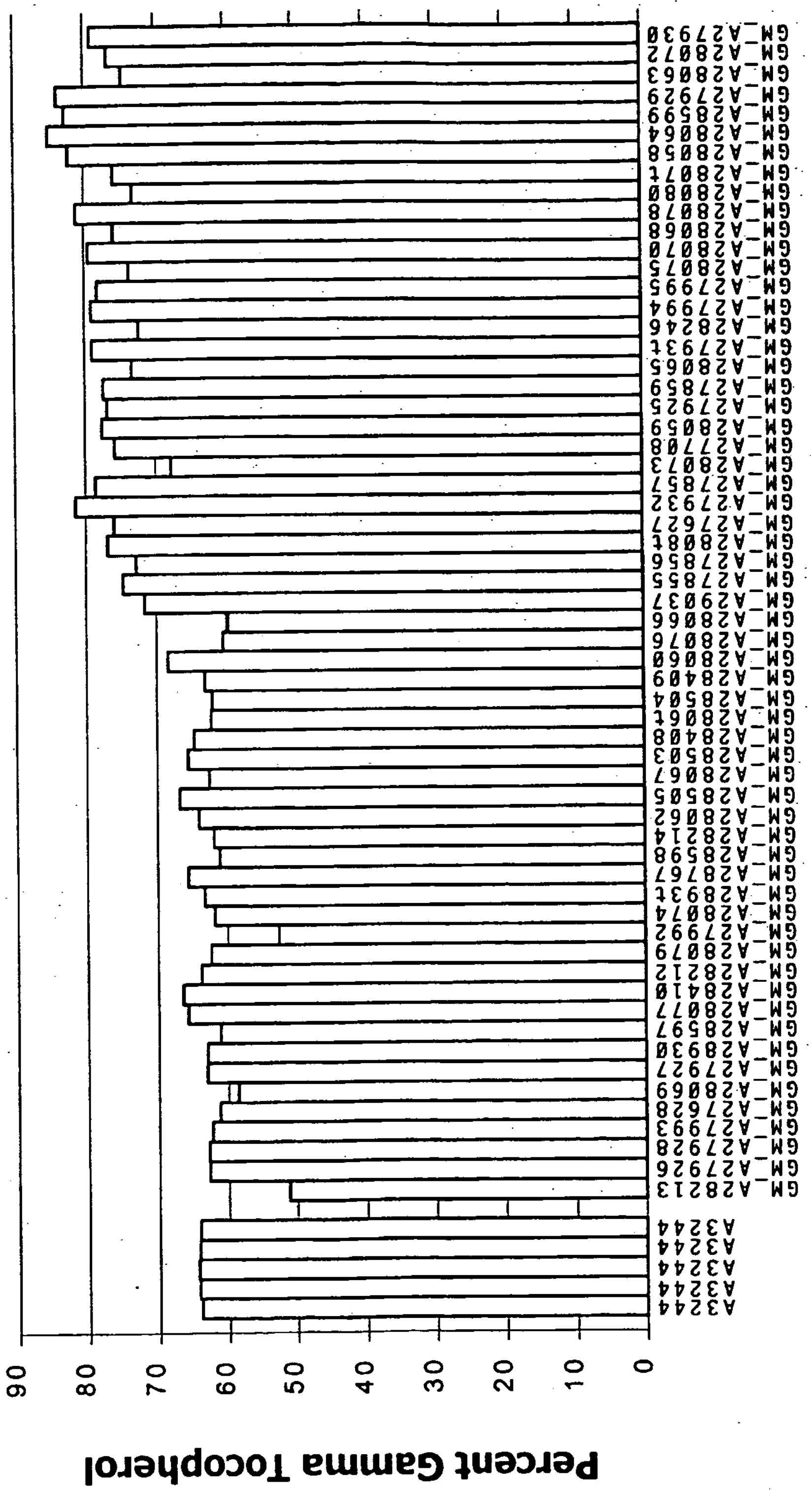
GM_A27927
GM_A28069
GM_A27628
GM_A27993
GM_A27928
GM_A27926
GM_A28213
A3244
A3244
A3244
A3244
A3244

Fig. 18b


Percent Gamma Tocopherol
0
10
20
30
40
50
60
70
80
90
A3244
A3244
A3244
A3244
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GM_A28213
GM_A27926
GM_A27928
GM_A27933
GM_A27628
GM_A28069
GM_A27927
GM_A28930
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GM_A28077
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GM_A27995
GM_A28075
GM_A28070
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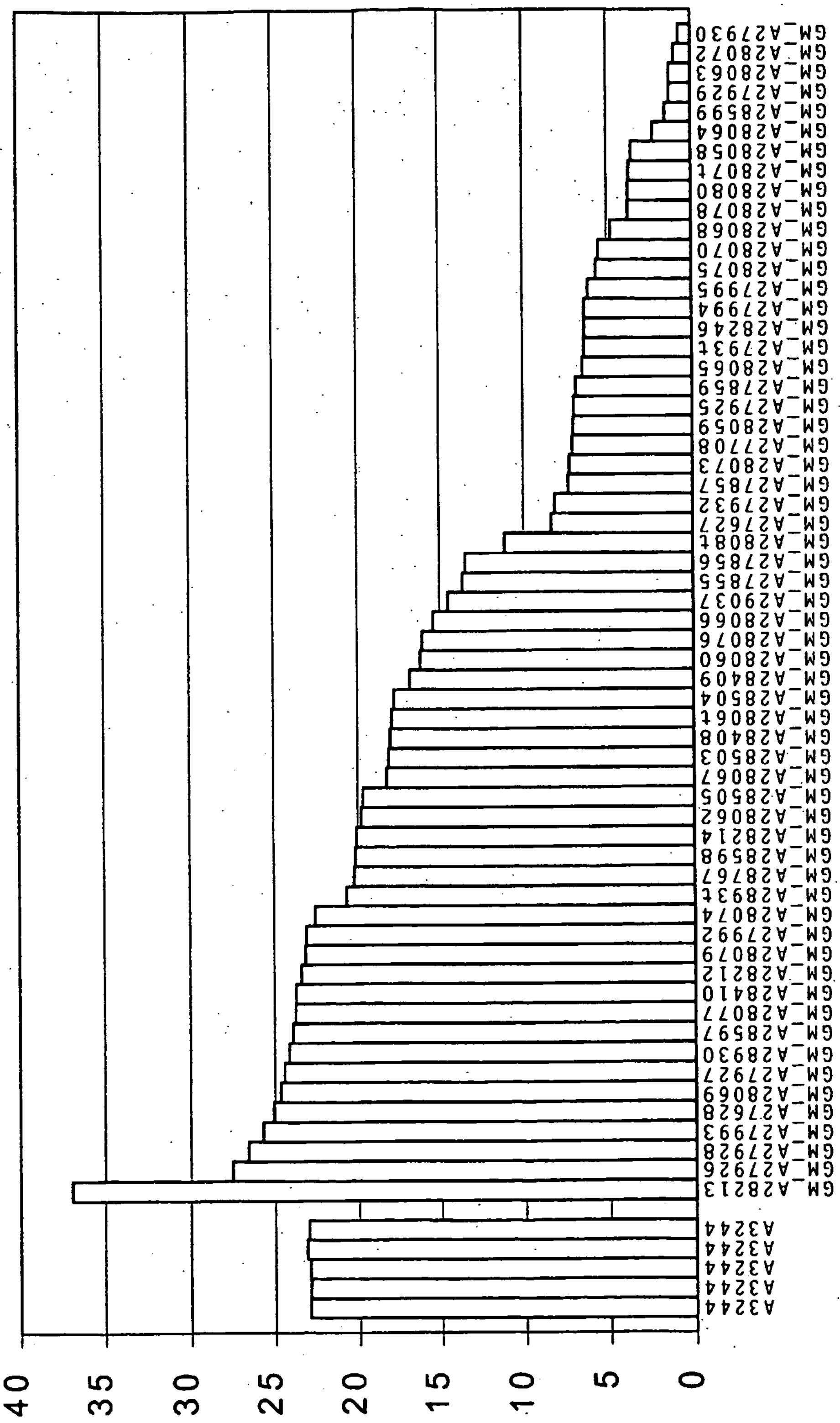
GM_A28080
GM_A28071
GM_A28058
GM_A28064
GM_A28599
GM_A27929
GM_A28063
GM_A28072
GM_A27930

Fig. 18c


0
5
10
15
20
25
30
35
40
A3244
A3244
A3244
A3244
A3244
GM_A28213
GM_A27926
GM_A27928
GM_A27993
GM_A27628
GM_A28069
GM_A27927
GM_A28930
GM_A28597
GM_A28077
GM_A28410
GM_A28212
GM_A28079
GM_A27992
GM_A28074
GM_A28931
GM_A28767
GM_A28598
GM_A28214
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GM_A28067
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GM_A28066
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GM_A27856
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GM_A27627
GM_A27932
GM_A27857
GM_A28073
GM_A27708
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GM_A27859
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GM_A28080
GM_A28071
GM_A28058
GM_A28064
GM_A28599
GM_A27929
GM_A28063
GM_A28072
GM_A27930

Fig. 18d

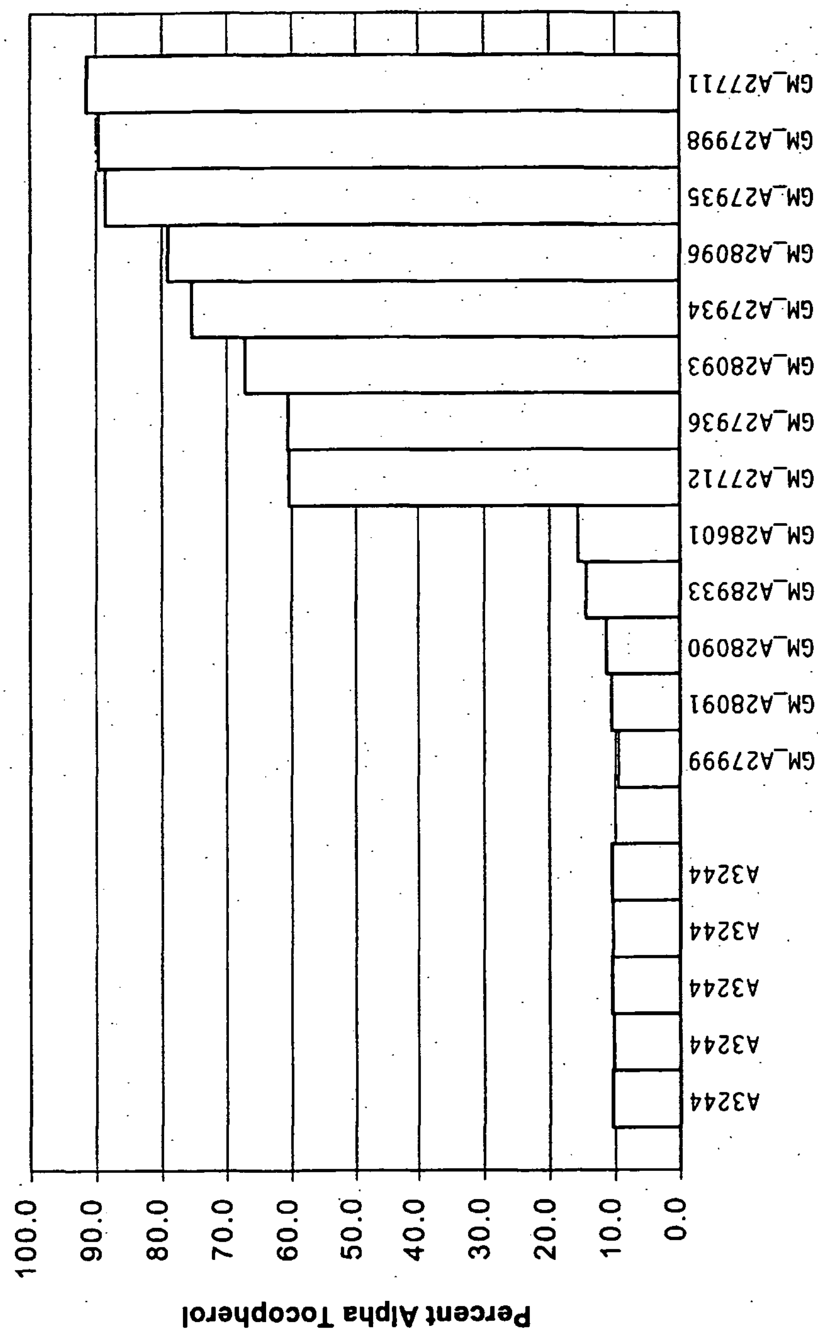
Percent Alpha Tocopherol
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
GM_A27711
GM_A27998
GM_A27935
GM_A28096
GM_A27934
GM_A28093
GM_A27936
GM_A27712
GM_A28601
GM_A28933
GM_A28090
GM_A28091
GM_A27999
A3244
A3244
A3244
A3244
A3244

Fig. 19a

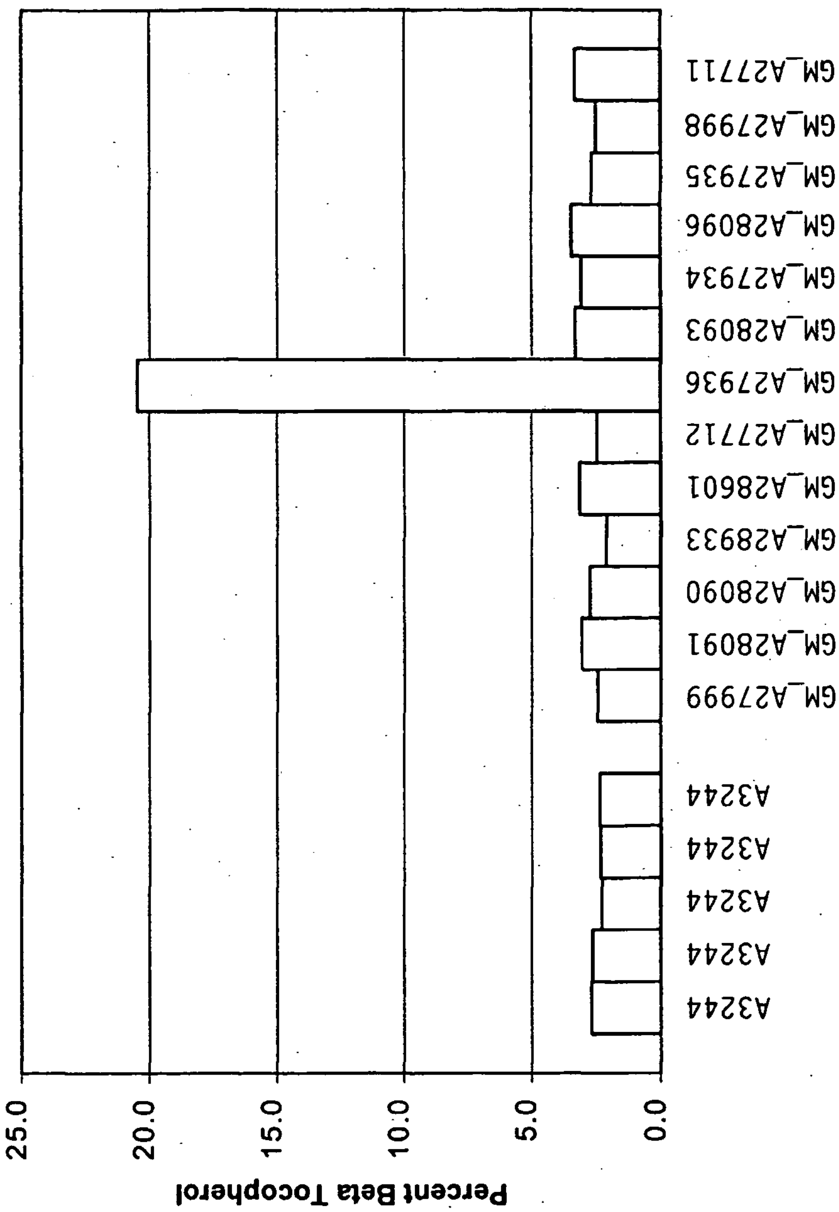
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Fig. 19b

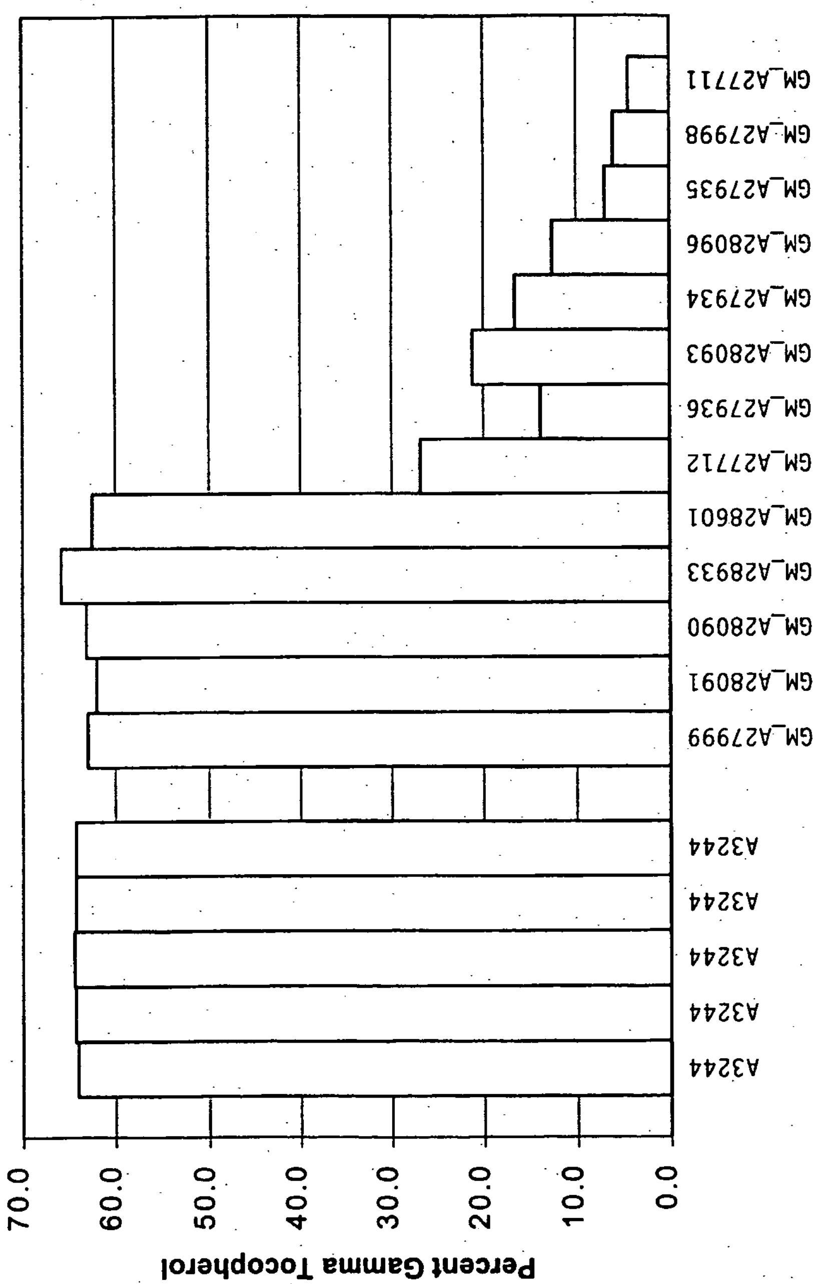
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Fig. 19c

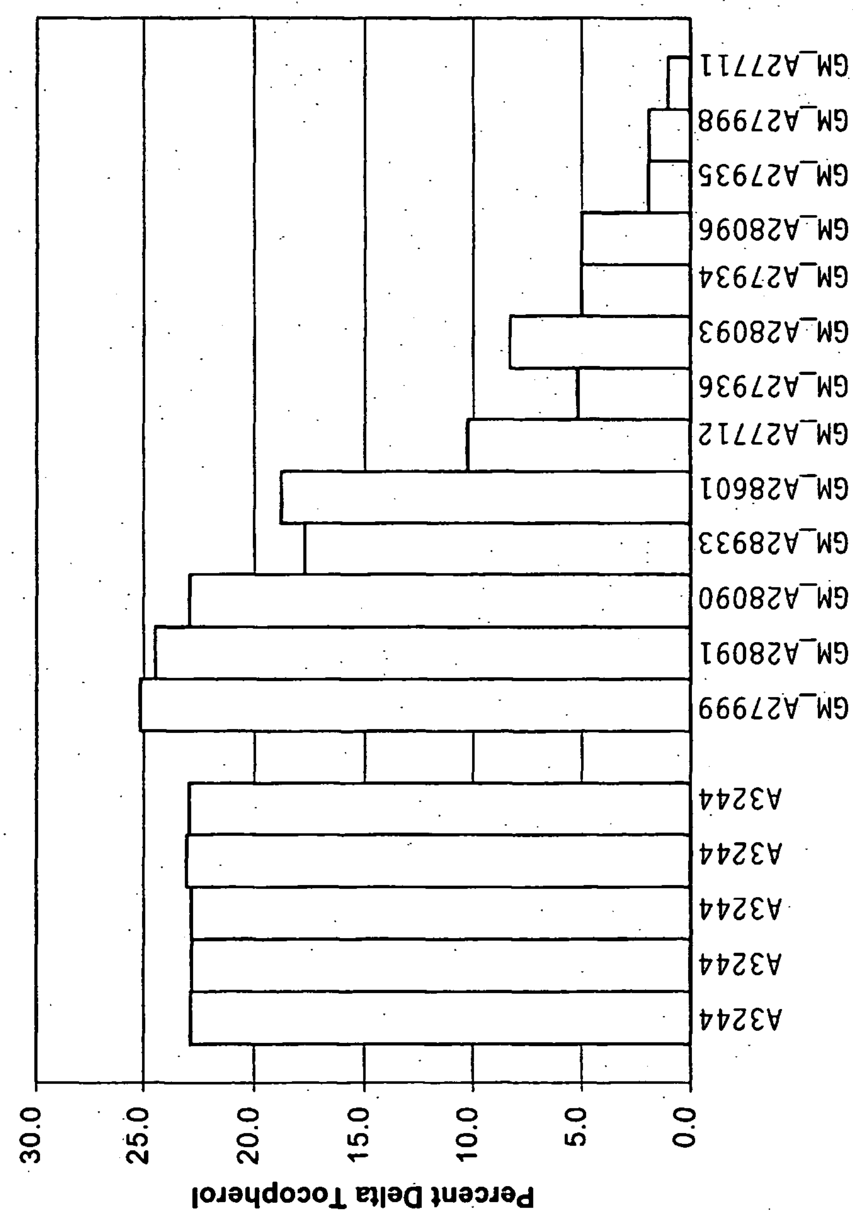
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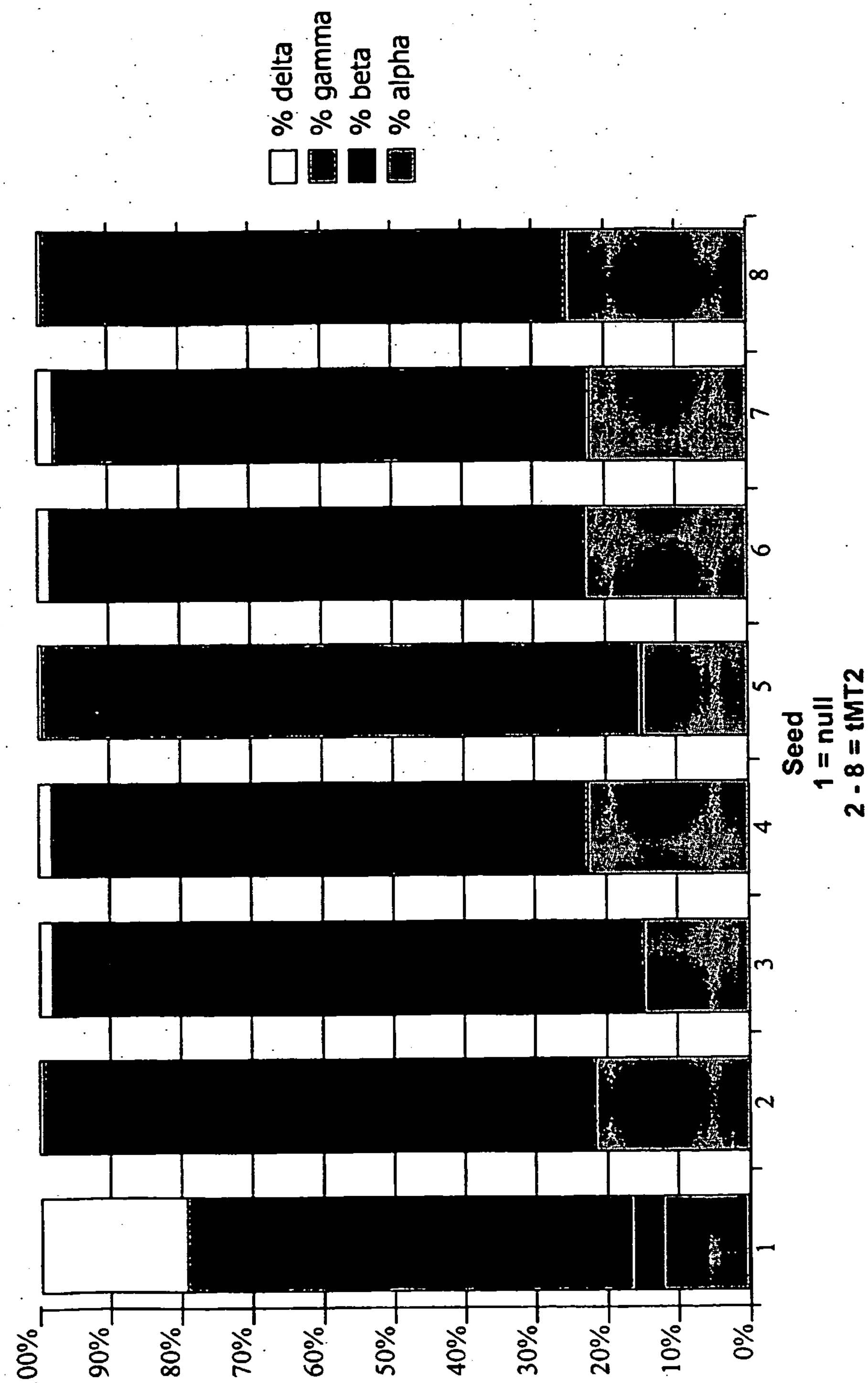
Fig. 19d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7
8
Seed
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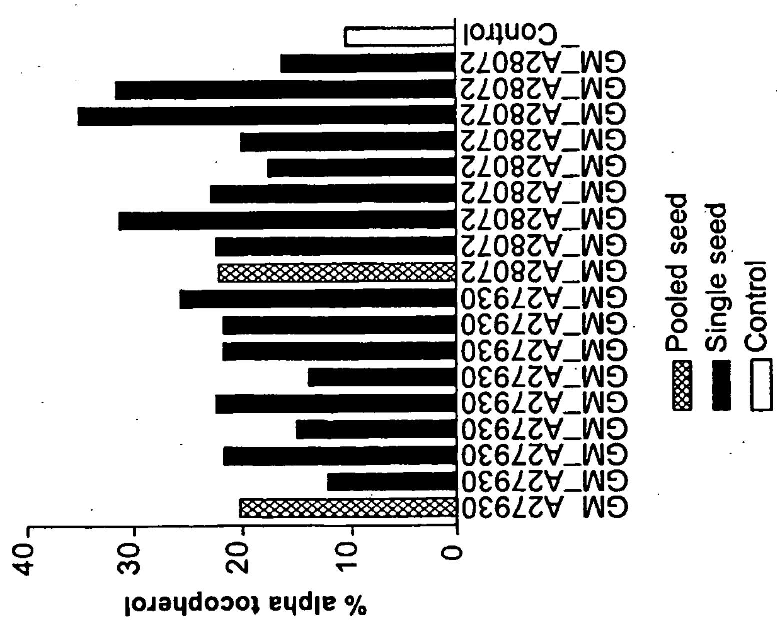
Fig. 20



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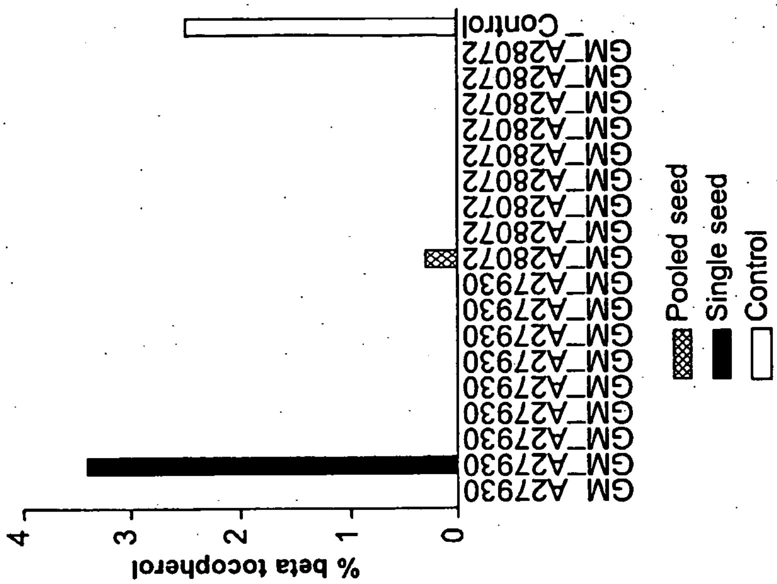

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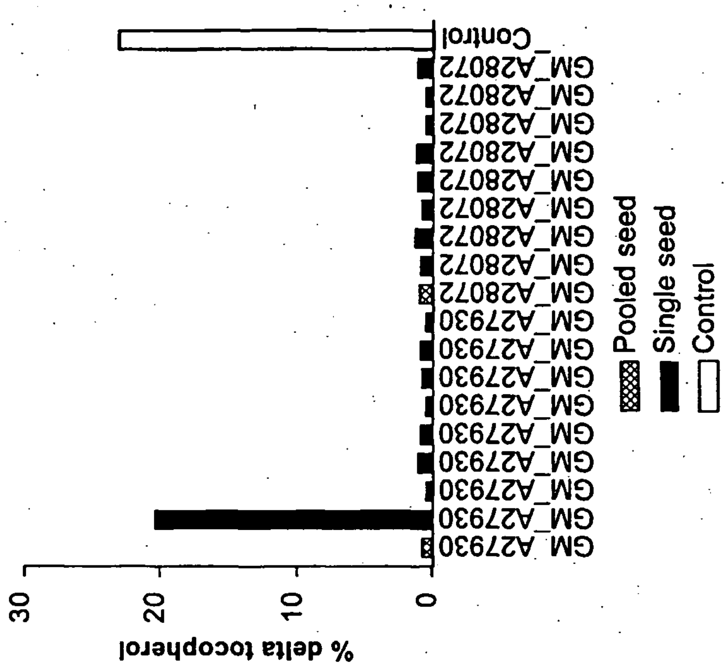
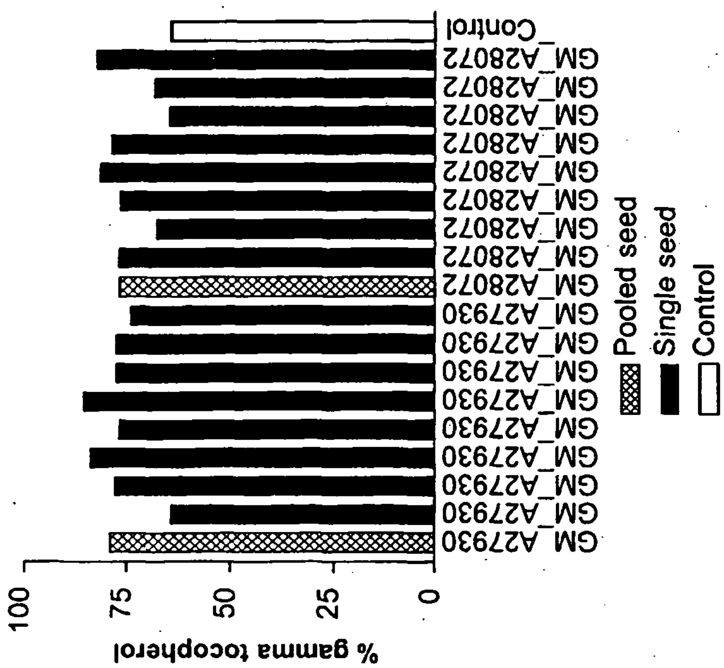


Fig. 21d



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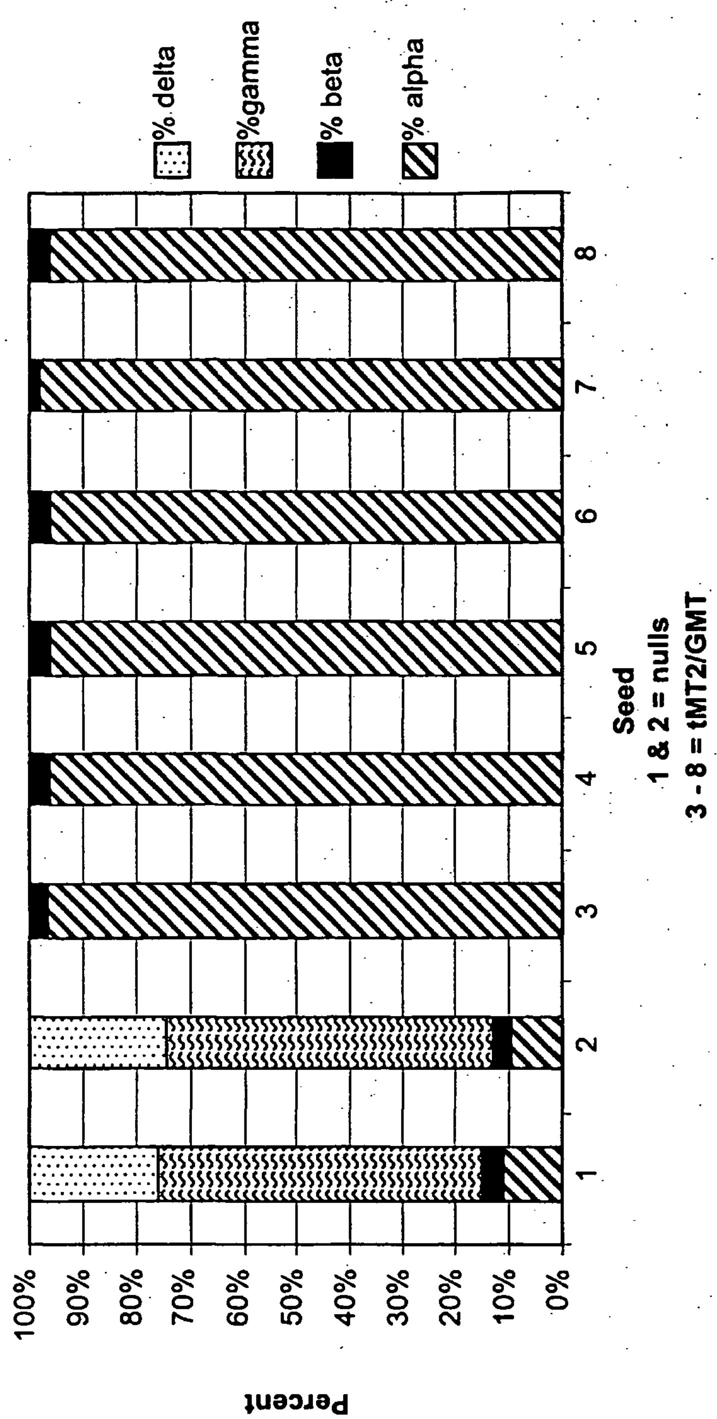
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30%
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2
3
4
5
6
7
8
Seed
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3 - 8 = tMT2/GMT

Fig. 22

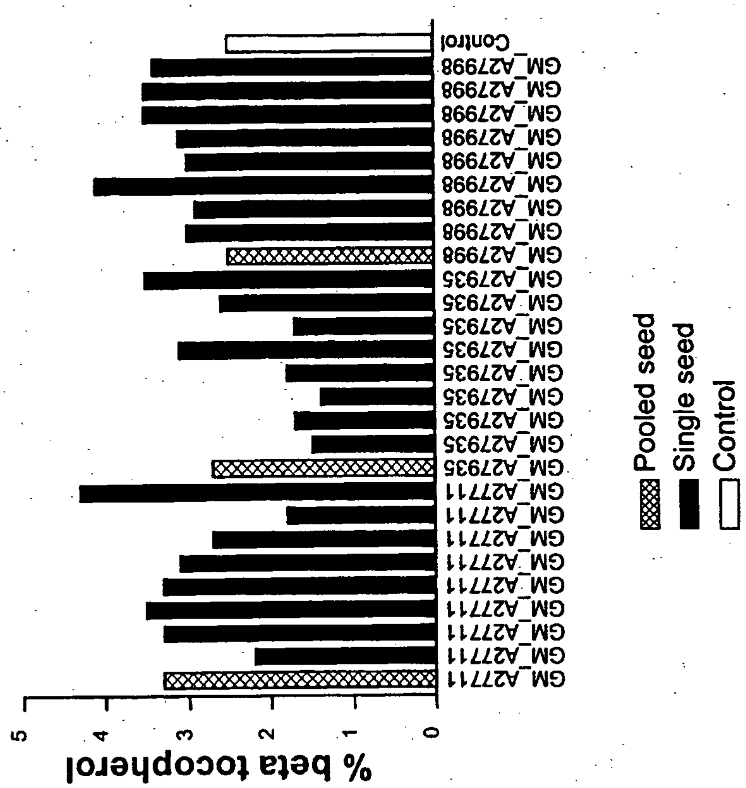
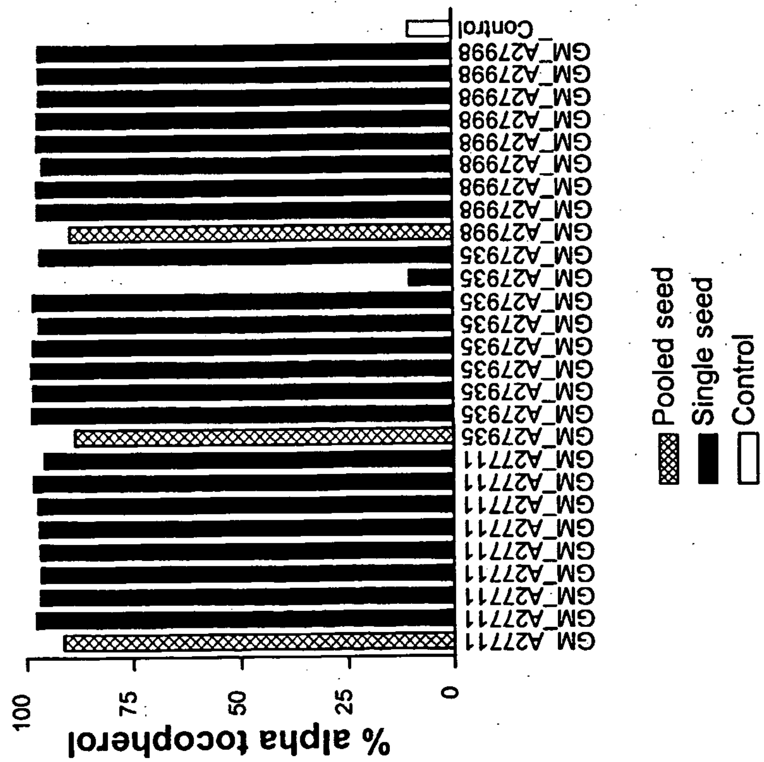


Fig.23b



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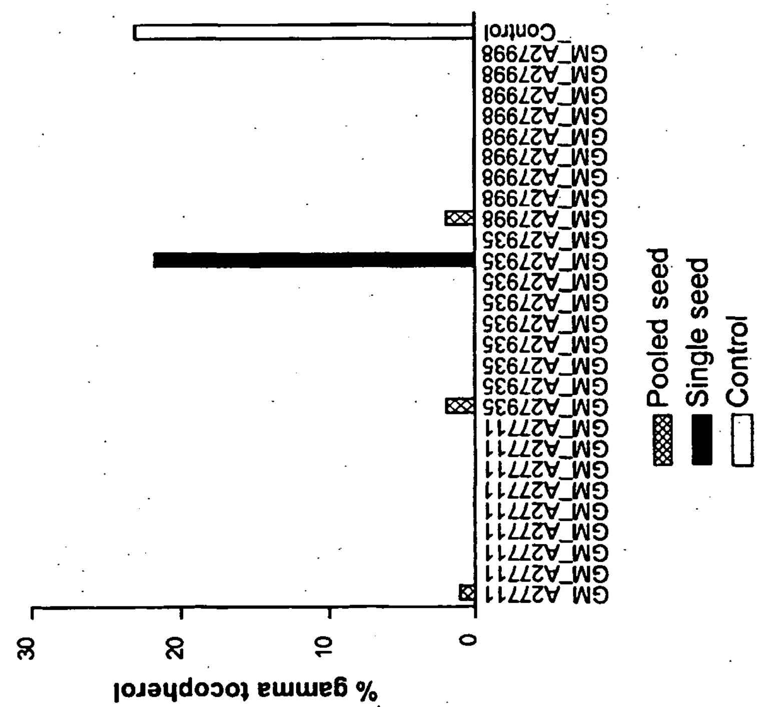

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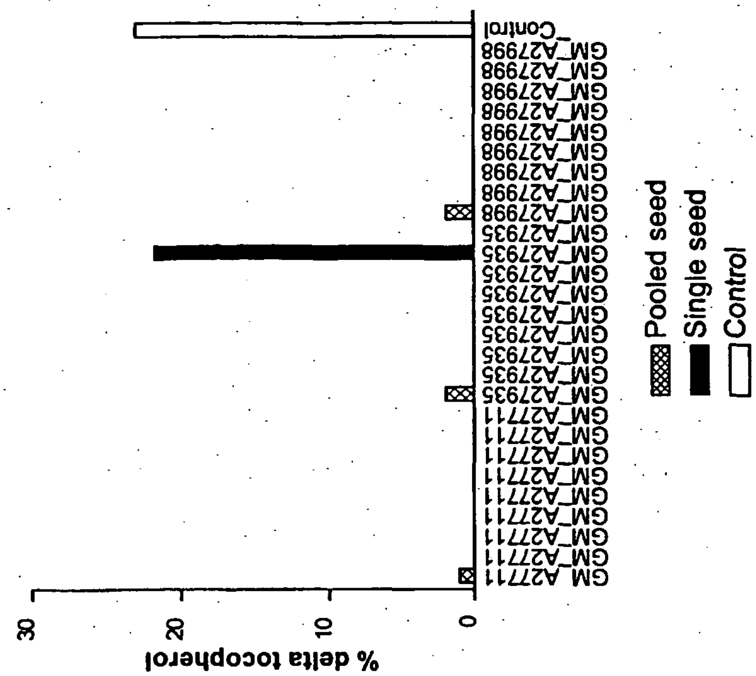

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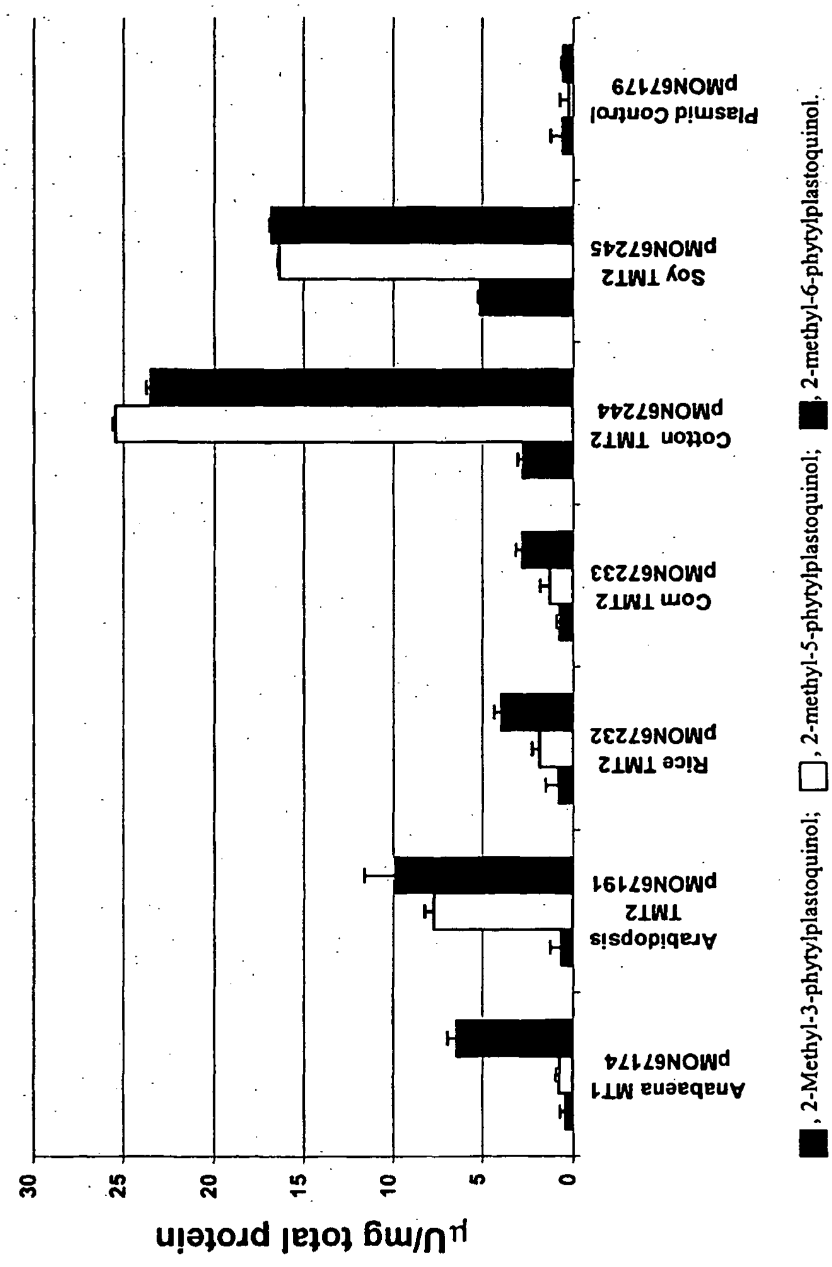
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Fig. 24

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Stein, Joshua
Norris, Susan

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tttagggatg tcaagctgaa gaggattgga ccgaagtggt accgcggtgt ccgaaggcat 840

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gagcttggtc ccaaggcgga ggatgtcagc aagccagtga atccgatcac cttcctcttc 960

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atctacagtc cgaagatgtc cagggtagga acgacgatag ccccgaggtg cagcttatca 180

gcgtcaaggc cagcttcaca accaagattc atacaacaca aaaaagaggc cttttggttc 240

tacaggttcc tctcaattgt ctatgaccat gtcataaacc caggtcactg gactgaagac 300

atgagggatg atgcacttga gccggctgat ctcaatgaca gggacatggt agttgtagat 360

gttggtggtg gaactggttt cactactttg ggtattgttc agcatgtgga tgctaagaat 420

gttacaatcc ttgaccaatc tcctcaccag cttgcaaagg ctaaacagaa ggagcctctc 480

aaggaatgca acataattga aggtgatgca gaagatcttc cttttcctac tgattatgcc 540

gatagatatg tgtctgctgg aagcatagag tactggccag acccacaacg ggggatcaag 600

gaagcataca gggtgttgaa acaaggagga aaagcttgct taattggtcc tgtgtaccct 660

acattttggt tgtctcgttt ctttgcagac gtttggatgc ttttccctaa ggaggaagaa 720

tatatagagt ggtttgaaaa ggctggattt aaggatgtcc aactcaaaag gattggccct 780

aaatggtatc gtggagttcg ccgacatggt ttgatcatgg ggtgctctgt aaccggtgtt 840

aaacccgcat ctggggactc tcctttgcag cttggaccta aggcagagga tgtatcaaag 900

ccggtaaatc cgtttgtatt tctcttacgc ttcatgttgg gtgccactgc agcagcatat 960

tatgtactgg ttcctatcta catgtggctc aaagatcaaa ttgtaccaga gggtcaacca 1020

atctaa 1026

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<212> DNA
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gcgtcctcat catcccgacc tgcttctcag ccgaggttca tccagcacaa gcaggaggcg 240

ttctggttct acaggttcct gtcgatagtg tacgaccatg tgataaaccc agggcactgg 300

accgaggaca tgagagacga tgcgttggag ccagccgagc tgtacgattc caggatgaag 360

gtggtggacg taggaggagg aactgggttc accaccttgg ggattataaa gcacatcgac 420

cctaaaaacg ttacgattct ggatcagtct ccgcatcagc ttgagaaggc taggcagaag 480

gaggctttga aggagtgtac tattgttgaa ggtgatgctg aggatctccc ttttcctact 540

gatactttcg atcgatatgt atctgctggc agcatagaat actggccaga cccacaaaga 600

gggataaagg aagcataccg ggttctaaaa ctgggaggcg ttgcctgctt gataggaccc 660

gtgcacccta ccttctggct ttccaggttc ttcgccgaca tgtggatgtt gttccccacc 720

gaagaagaat acatagagtg gtttaaaaag gccgggttca aagatgtgaa gttgaagagg 780

attggcccaa aatggtaccg tggtgtgcgt agacacgggc tcatcatggg ctgttccgtc 840

actggtgtta aacgtctctc tggtgactcc cctcttcagc ttggaccgaa ggcggaggat 900

gtgaagaagc cgatcaatcc attctcgttc cttctgcgct tcattttggg tacgatagca 960

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ggccagccca tatga 1035

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<211> 1029
<212> DNA
<213> Arabidopsis thaliana

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gctaccacta gtgctaaagt tcccaacttt agaagcatag tagtacccaa gtgtagtgtc 180

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gacatgaggg atgatgccct tgaacccgct gatctcaatg acaggaacat gattgtggtg    360
gatgttggtg gcggcacggg tttcaccact cttggtattg tcaagcacgt ggatgccaag    420
aatgtcacca ttcttgacca gtcaccccac cagctcgcca aggccaagca gaaggagcca    480
ctcaaggaat gcaaaataat cgaaggggat gccgaggatc tcccctttcg aactgattat    540
gccgatagat atgtatccgc aggaagtatt gagtactggc cggatccaca gcgtggcatc    600
aaggaggcat acagggtttt gaaacttgga ggcaaagcgt gtctaattgg tccggtctac    660
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gagtatattg agtggtttca gaaggcaggg tttaaggacg tccaactaaa aaggattggc    780
ccaaaatggt atcgtggggt tcgccgtcat ggcttgatta tgggttgttc agtgaccggt    840
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aagcccgtta atccttttgt ctttgcactg cgcttcgttt tgggtgcctt ggcagcgaca    960
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<213>  Arabidopsis thaliana

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ctcgtgctca ccaggcgtgg gggaggcggc ggcaacatct ccgtggctcg gctgaggtgc    180
gcggcgtcgt cgtcgtcggc ggcggcgagg ccgatgtcgc agccgcggtt catccagcac    240
aagaaggagg cgttctggtt ctaccgcttc ctctccatcg tctacgacca cgtcatcaac    300
ccgggccact ggacggagga catgcgggac gacgccctcg agcccgccga cctctacagc    360
cgcaagctca gggtcgtcga cgtcggcggc gggacggggt tcaccacgct cgggatcgtc    420
aagcgcgtcg acccggagaa cgtcacgctg ctcgaccagt ccccgcacca gctcgagaag    480
gcccgggaga aggaggccct caagggcgtc accatcatgg agggcgacgc cgaggacctc    540
cccttcccca ccgacacctt cgaccgctac gtctccgccg gcagcatcga gtattggccc    600
gatccgcagc gaggaatcaa ggaagcttac agggttttga ggcttggtgg agtggcttgc    660
atgattggcc ccgtgcaccc aaccttctgg ctgtctcgct tttcgctga catgtggatg    720
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ctcttcccga aggaagagga gtatattgag tggttcaaaa aggcagggtt caaggatgtc 780

aagctcaaaa ggattggacc aaaatggtac cgtggtgtcc gaaggcatgg cctgattatg 840

ggatgctctg tgacgggcgt caaaagagaa catggagact cccctttgca gcttggtcca 900

aaggttgagg atgtcagcaa acctgtgaat cctatcacct tcctcttccg cttcctcatg 960

ggaacaatat gtgctgcata ctatgttctg gtgcctatct acatgtggat aaaggaccag 1020

attgtgccca aaggcatgcc gatctaa 1047

<210> 13
<211> 1014
<212> DNA
<213> Arabidopsis thaliana

<400> 13
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tccaatctac acgccagacc aagtcctccg ctgagtctcg tctcaaacac agccacgcgg 120

agactctccg tggcgacaag atgcagcagc agcagcagcg tgtcggcgtc aaggccatct 180

gcgcagccta ggttcatcca gcacaagaaa gaggcctact ggttctacag gttcctgtcc 240

atcgtgtacg accacatcat caatcccggc cactggacgg aggatatgag ggacgacgct 300

ctcgagcctg cggatctgag ccatccggac atgcgagttg tcgacgtcgg aggcggaacg 360

ggtttcacca cgctgggaat cgtcaagacg gtgaaggcta agaacgtgac gattctggac 420

cagtcgccgc atcagctggc aaaggcgaag cagaaggagc cgttgaagga gtgcaagatc 480

gttgaaggag atgcggagga tctccctttt cctactgatt atgctgacag atacgtctct 540

gctggaagca ttgagtactg gcccgacccg cagagggggа taagggaagc gtacagagtt 600

ctcaagatcg gtgggaaagc atgtctcatt ggccctgtcc acccgacgtt ttggctttct 660

cgtttctttg cagatgtgtg gatgcttttc cccaaggagg aggagtacat tgagtggttc 720

aagaatgctg gtttcaagga cgttcagctt aagaggattg gccccaagtg gtaccgtggt 780

gttcgcaggc acggacttat catgggatgc tctgttactg gtgtcaaacc tgcctctgga 840

gactctcctc tccagcttgg accaaaggaa gaggacgtgg agaagcctgt aaacaatcct 900

ttctccttct tgggacgctt cctcttggga accttagcgg ctgcctggtt tgtgttaatc 960

ccaatctaca tgtggatcaa ggatcagatc gttcccaaag accaacccat ctga 1014

<210> 14
<211> 1014
<212> DNA
<213> Arabidopsis thaliana

<400> 14

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agactctccg tggcgacaag atgcagcagc agcagcagcg tgtcggcgtc aaggccatct    180
gcgcagccta ggttcatcca gcacaagaaa gaggcctact ggttctacag gttcctgtcc    240
atcgtgtacg accacatcat caatcccggc cactggacgg aggatatgag ggacgacgct    300
ctcgagcctg cggatctgag ccatccggac atgcgagttg tcgacgtcgg aggcggaacg    360
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cagtcgccgc atcagctggc aaaggcgaag cagaaggagc cgttgaagga gtgcaagatc    480
gtggaaggag atgcggagga tctccctttt cctactgatt atgctgacag atacgtctct    540
gctggaagca ttgagtactg gcccgacccg cagaggggta taagggaagc gtacagagtt    600
ctcaagatcg gtgggaaagc atgtctcatt ggccctgtcc acccgacgtt ttggctttca    660
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aagaatgctg gtttcaagga cgttcagctt aagaggattg gccccaagtg gtaccgtggt    780
gttcgcaggc acggacttat catgggatgc tctgttactg gtgtcaaacc tgcctctgga    840
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ttctccttct tgggacgctt cctcttgggt accctagcgg ctgcctggtt tgtgttaatc    960
ccaatctaca tgtggatcaa ggatcagatc gttcccaaag accaacccat ctga         1014
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<210>  15
<211>  1017
<212>  DNA
<213>  Arabidopsis thaliana

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acacctagac tctcggtggc tactagatgc agcagcagca gcgtgtcgtc ttcccggcca    180
tcggcgcaac ctaggttcat tcagcacaag aaggaggctt actggttcta caggttctta    240
tccatcgtat acgaccatgt catcaatcct gggcattgga ccgaggatat gagagacgac    300
gctcttgagc cagcggatct cagccatccg gacatgcgag tggtcgatgt cggcggcgga    360
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gaccagtcgc cacatcagct ggccaaagca aagcaaaagg agccgttgaa agaatgcaag    480
atcgtcgagg gagatgctga ggatcttcct tttccaaccg attatgctga cagatacgtt    540
tctgctggaa gcattgagta ctggccggac ccgcagaggg gaataaggga agcgtacagg    600
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gttctcaaga tcggtggcaa agcgtgtctc atcggccctg tctacccaac cttctggctc 660

tctcgcttct tttctgatgt ctggatgctc ttccccaagg aggaagagta cattgagtgg 720

ttcaagaatg ccggtttcaa ggacgttcag ctcaagagga ttggccccaa gtggtaccgt 780

ggtgttcgca ggcacggcct tatcatggga tgttctgtca ctggtgttaa acctgcctcc 840

ggtgactctc ctctccagct tggtccaaag gaagaggacg tagagaagcc tgtcaacaac 900

cccttctcct tcttgggacg cttcctcctg ggaactctag cagctgcctg gtttgtgtta 960

atccctatct acatgtggat caaggatcag atcgttccca aagaccaacc catctga 1017

<210> 16
<211> 338
<212> PRT
<213> Arabidopsis thaliana

<400> 16

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
325 330 335

Pro Ile

<210> 17
<211> 338
<212> PRT
<213> Arabidopsis thaliana

<400> 17

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr

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            20                  25                  30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
        35                  40                  45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
    50                  55                  60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
65                  70                  75                  80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
                85                  90                  95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
            100                 105                 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
        115                 120                 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
    130                 135                 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145                 150                 155                 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
                165                 170                 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
            180                 185                 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
        195                 200                 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
    210                 215                 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225                 230                 235                 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
                245                 250                 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
            260                 265                 270
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Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
        275                 280                 285

Pro Lys Glu Lys Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
    290                 295                 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305                 310                 315                 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
                325                 330                 335

Pro Ile


<210>  18
<211>  338
<212>  PRT
<213>  Arabidopsis thaliana

<400>  18

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1               5                   10                  15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
            20                  25                  30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
        35                  40                  45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
    50                  55                  60

Arg Phe Ile Gln His Lys Lys Lys Ala Tyr Trp Phe Tyr Arg Phe Leu
65                  70                  75                  80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
                85                  90                  95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
            100                 105                 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
        115                 120                 125
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Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
    130                 135                 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145                 150                 155                 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
                165                 170                 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
            180                 185                 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
        195                 200                 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
    210                 215                 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225                 230                 235                 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
                245                 250                 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
            260                 265                 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
        275                 280                 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
    290                 295                 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305                 310                 315                 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
                325                 330                 335

Pro Ile
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<210>  19
<211>  338
<212>  PRT
<213>  Arabidopsis thaliana
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<400> 19

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Ser Lys Gly Leu
1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
100 105 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
325 330 335

Pro Ile

<210> 20
<211> 338
<212> PRT
<213> Arabidopsis thaliana

<400> 20

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1 5 10 15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
20 25 30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
35 40 45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
50 55 60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
65 70 75 80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
85 90 95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met

100 105 110

Arg Val Val Asn Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
115 120 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
130 135 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145 150 155 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
165 170 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
180 185 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
195 200 205

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
210 215 220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225 230 235 240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
245 250 255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
260 265 270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
275 280 285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
290 295 300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305 310 315 320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
325 330 335

Pro Ile

<210> 21
<211> 338
<212> PRT
<213> Arabidopsis thaliana

<400> 21

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Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1               5                   10                  15

Gly Ser Pro Gly Ser Asn Leu His Ala Arg Ser Ile Pro Arg Pro Thr
            20                  25                  30

Leu Leu Ser Val Thr Arg Thr Ser Thr Pro Arg Leu Ser Val Ala Thr
        35                  40                  45

Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala Gln Pro
    50                  55                  60

Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu
65                  70                  75                  80

Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Ile Glu Asp
                85                  90                  95

Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met
            100                 105                 110

Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
        115                 120                 125

Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
    130                 135                 140

His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys
145                 150                 155                 160

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala
                165                 170                 175

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
            180                 185                 190

Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala
        195                 200                 205
```

Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe
    210   215   220

Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp
225   230   235   240

Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro
                245   250   255

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
            260   265   270

Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly
        275   280   285

Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe
    290   295   300

Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu
305   310   315   320

Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln
                325   330   335

Pro Ile

<210> 22
<211> 352
<212> PRT
<213> Arabidopsis thaliana

<400> 22

Met Ala Met Ala Ser Thr Tyr Ala Pro Gly Gly Gly Ala Arg Ala Leu
1   5   10   15

Ala Gln Gly Arg Cys Arg Val Arg Gly Pro Ala Gly Leu Gly Phe Leu
            20   25   30

Gly Pro Ser Lys Ala Ala Gly Leu Pro Arg Pro Leu Ala Leu Ala Leu
        35   40   45

Ala Arg Arg Met Ser Ser Pro Val Ala Val Gly Ala Arg Leu Arg Cys
    50   55   60

Ala Ala Ser Ser Ser Pro Ala Ala Ala Arg Pro Ala Thr Ala Pro Arg
65   70   75   80

```
Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser
                85                  90                  95

Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
            100                 105                 110

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Phe Ser Arg His Leu Thr
        115                 120                 125

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
    130                 135                 140

Lys His Val Asn Pro Glu Asn Val Thr Leu Leu Asp Gln Ser Pro His
145                 150                 155                 160

Gln Leu Asp Lys Ala Arg Gln Lys Glu Ala Leu Lys Gly Val Thr Ile
                165                 170                 175

Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Ser Phe Asp
            180                 185                 190

Arg Tyr Ile Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
        195                 200                 205

Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Phe Gly Gly Leu Ala Cys
    210                 215                 220

Val Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
225                 230                 235                 240

Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
                245                 250                 255

Lys Lys Ala Gly Phe Arg Asp Val Lys Leu Lys Arg Ile Gly Pro Lys
            260                 265                 270

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
        275                 280                 285

Thr Gly Val Lys Arg Glu Arg Gly Asp Ser Pro Leu Glu Leu Gly Pro
    290                 295                 300

Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Ile Thr Phe Leu Phe
305                 310                 315                 320
```

Arg Phe Leu Val Gly Thr Ile Cys Ala Ala Tyr Tyr Val Leu Val Pro
325 330 335

Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Gly Met Pro Ile
340 345 350

<210> 23
<211> 341
<212> PRT
<213> Arabidopsis thaliana

<400> 23

Met Ala Ser Ser Met Leu Asn Gly Ala Glu Thr Phe Thr Leu Ile Arg
1 5 10 15

Gly Val Thr Pro Lys Ser Ile Gly Phe Leu Gly Ser Gly Leu His Gly
20 25 30

Lys Gln Phe Ser Ser Ala Gly Leu Ile Tyr Ser Pro Lys Met Ser Arg
35 40 45

Val Gly Thr Thr Ile Ala Pro Arg Cys Ser Leu Ser Ala Ser Arg Pro
50 55 60

Ala Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe
65 70 75 80

Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His
85 90 95

Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn
100 105 110

Asp Arg Asp Met Val Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr
115 120 125

Thr Leu Gly Ile Val Gln His Val Asp Ala Lys Asn Val Thr Ile Leu
130 135 140

Asp Gln Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu
145 150 155 160

Lys Glu Cys Asn Ile Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro
165 170 175

Thr Asp Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp

180 185 190

Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Gln
195 200 205

Gly Gly Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu
210 215 220

Ser Arg Phe Phe Ala Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu
225 230 235 240

Tyr Ile Glu Trp Phe Glu Lys Ala Gly Phe Lys Asp Val Gln Leu Lys
245 250 255

Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile
260 265 270

Met Gly Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro
275 280 285

Leu Gln Leu Gly Pro Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro
290 295 300

Phe Val Phe Leu Leu Arg Phe Met Leu Gly Ala Thr Ala Ala Ala Tyr
305 310 315 320

Tyr Val Leu Val Pro Ile Tyr Met Trp Leu Lys Asp Gln Ile Val Pro
325 330 335

Glu Gly Gln Pro Ile
340

<210> 24
<211> 344
<212> PRT
<213> Arabidopsis thaliana

<400> 24

Met Ala Ser Ser Met Leu Ser Gly Ala Glu Ser Leu Ser Met Leu Arg
1 5 10 15

Ile His His Gln Pro Lys Leu Thr Phe Ser Ser Pro Ser Leu His Ser
20 25 30

Lys Pro Thr Asn Leu Lys Met Asp Leu Ile Pro Phe Ala Thr Lys His
35 40 45

```
Gln Lys Thr Lys Lys Ala Ser Ile Phe Thr Cys Ser Ala Ser Ser Ser
    50                  55                  60

Ser Arg Pro Ala Ser Gln Pro Arg Phe Ile Gln His Lys Gln Glu Ala
65                  70                  75                  80

Phe Trp Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn
                85                  90                  95

Pro Gly His Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala
            100                 105                 110

Glu Leu Tyr Asp Ser Arg Met Lys Val Val Asp Val Gly Gly Gly Thr
        115                 120                 125

Gly Phe Thr Thr Leu Gly Ile Ile Lys His Ile Asp Pro Lys Asn Val
    130                 135                 140

Thr Ile Leu Asp Gln Ser Pro His Gln Leu Glu Lys Ala Arg Gln Lys
145                 150                 155                 160

Glu Ala Leu Lys Glu Cys Thr Ile Val Glu Gly Asp Ala Glu Asp Leu
                165                 170                 175

Pro Phe Pro Thr Asp Thr Phe Asp Arg Tyr Val Ser Ala Gly Ser Ile
            180                 185                 190

Glu Tyr Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val
        195                 200                 205

Leu Lys Leu Gly Gly Val Ala Cys Leu Ile Gly Pro Val His Pro Thr
    210                 215                 220

Phe Trp Leu Ser Arg Phe Phe Ala Asp Met Trp Met Leu Phe Pro Thr
225                 230                 235                 240

Glu Glu Glu Tyr Ile Glu Trp Phe Lys Lys Ala Gly Phe Lys Asp Val
                245                 250                 255

Lys Leu Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His
            260                 265                 270

Gly Leu Ile Met Gly Cys Ser Val Thr Gly Val Lys Arg Leu Ser Gly
        275                 280                 285
```

```
Asp Ser Pro Leu Gln Leu Gly Pro Lys Ala Glu Asp Val Lys Lys Pro
    290                 295                 300

Ile Asn Pro Phe Ser Phe Leu Leu Arg Phe Ile Leu Gly Thr Ile Ala
305                 310                 315                 320

Ala Thr Tyr Tyr Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln
                325                 330                 335

Ile Val Pro Lys Gly Gln Pro Ile
            340
```

<210> 25
<211> 342
<212> PRT
<213> Arabidopsis thaliana

<400> 25

```
Met Gly Ser Val Met Leu Ser Gly Thr Glu Lys Leu Thr Leu Arg Thr
1               5                   10                  15

Leu Thr Gly Asn Gly Leu Gly Phe Thr Gly Ser Asp Leu His Gly Lys
            20                  25                  30

Asn Phe Pro Arg Val Ser Phe Ala Ala Thr Thr Ser Ala Lys Val Pro
        35                  40                  45

Asn Phe Arg Ser Ile Val Val Pro Lys Cys Ser Val Ser Ala Ser Arg
    50                  55                  60

Pro Ser Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp
65                  70                  75                  80

Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly
                85                  90                  95

His Trp Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu
            100                 105                 110

Asn Asp Arg Asn Met Ile Val Val Asp Val Gly Gly Gly Thr Gly Phe
        115                 120                 125

Thr Thr Leu Gly Ile Val Lys His Val Asp Ala Lys Asn Val Thr Ile
    130                 135                 140

Leu Asp Gln Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro
145                 150                 155                 160
```

Leu Lys Glu Cys Lys Ile Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe
165 170 175

Arg Thr Asp Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr
180 185 190

Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys
195 200 205

Leu Gly Gly Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp
210 215 220

Leu Ser Arg Phe Phe Ala Asp Val Trp Met Leu Phe Pro Lys Glu Glu
225 230 235 240

Glu Tyr Ile Glu Trp Phe Gln Lys Ala Gly Phe Lys Asp Val Gln Leu
245 250 255

Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu
260 265 270

Ile Met Gly Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser
275 280 285

Pro Leu Gln Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn
290 295 300

Pro Phe Val Phe Ala Leu Arg Phe Val Leu Gly Ala Leu Ala Ala Thr
305 310 315 320

Trp Phe Val Leu Val Pro Ile Tyr Met Trp Leu Lys Asp Gln Val Val
325 330 335

Pro Lys Gly Gln Pro Ile
340

<210> 26
<211> 348
<212> PRT
<213> Arabidopsis thaliana

<400> 26

Met Ala Met Ala Ser Ser Ala Tyr Ala Pro Ala Gly Gly Val Gly Thr
1 5 10 15

```
His Ser Ala Pro Gly Arg Ile Arg Pro Pro Arg Gly Leu Gly Phe Ser
            20                  25                  30

Thr Thr Thr Thr Lys Ser Arg Pro Leu Val Leu Thr Arg Arg Gly Gly
        35                  40                  45

Gly Gly Gly Asn Ile Ser Val Ala Arg Leu Arg Cys Ala Ala Ser Ser
    50                  55                  60

Ser Ser Ala Ala Ala Arg Pro Met Ser Gln Pro Arg Phe Ile Gln His
65                  70                  75                  80

Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser Ile Val Tyr Asp
                85                  90                  95

His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met Arg Asp Asp Ala
            100                 105                 110

Leu Glu Pro Ala Asp Leu Tyr Ser Arg Lys Leu Arg Val Val Asp Val
        115                 120                 125

Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val Lys Arg Val Asp
    130                 135                 140

Pro Glu Asn Val Thr Leu Leu Asp Gln Ser Pro His Gln Leu Glu Lys
145                 150                 155                 160

Ala Arg Glu Lys Glu Ala Leu Lys Gly Val Thr Ile Met Glu Gly Asp
                165                 170                 175

Ala Glu Asp Leu Pro Phe Pro Thr Asp Thr Phe Asp Arg Tyr Val Ser
            180                 185                 190

Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg Gly Ile Lys Glu
        195                 200                 205

Ala Tyr Arg Val Leu Arg Leu Gly Gly Val Ala Cys Met Ile Gly Pro
    210                 215                 220

Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala Asp Met Trp Met
225                 230                 235                 240

Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe Lys Lys Ala Gly
                245                 250                 255

Phe Lys Asp Val Lys Leu Lys Arg Ile Gly Pro Lys Trp Tyr Arg Gly
```

260 265 270

Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val Thr Gly Val Lys
275 280 285

Arg Glu His Gly Asp Ser Pro Leu Gln Leu Gly Pro Lys Val Glu Asp
290 295 300

Val Ser Lys Pro Val Asn Pro Ile Thr Phe Leu Phe Arg Phe Leu Met
305 310 315 320

Gly Thr Ile Cys Ala Ala Tyr Tyr Val Leu Val Pro Ile Tyr Met Trp
325 330 335

Ile Lys Asp Gln Ile Val Pro Lys Gly Met Pro Ile
340 345

<210> 27
<211> 337
<212> PRT
<213> Arabidopsis thaliana

<400> 27

Met Ala Ser Leu Met Leu Asn Gly Ala Ile Thr Phe Pro Lys Gly Leu
1 5 10 15

Gly Phe Pro Ala Ser Asn Leu His Ala Arg Pro Ser Pro Pro Leu Ser
20 25 30

Leu Val Ser Asn Thr Ala Thr Arg Arg Leu Ser Val Ala Thr Arg Cys
35 40 45

Ser Ser Ser Ser Ser Val Ser Ala Ser Arg Pro Ser Ala Gln Pro Arg
50 55 60

Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg Phe Leu Ser
65 70 75 80

Ile Val Tyr Asp His Ile Ile Asn Pro Gly His Trp Thr Glu Asp Met
85 90 95

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro Asp Met Arg
100 105 110

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
115 120 125

Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
130 135 140

Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys Ile
145 150 155 160

Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala Asp
165 170 175

Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
180 185 190

Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly Lys Ala Cys
195 200 205

Leu Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
210 215 220

Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
225 230 235 240

Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
245 250 255

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
260 265 270

Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
275 280 285

Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe Ser Phe Leu
290 295 300

Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe Val Leu Ile
305 310 315 320

Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Asp Gln Pro
325 330 335

Ile

<210> 28
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 28

```
Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1               5                   10                  15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
            20                  25                  30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
        35                  40                  45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
    50                  55                  60

Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65                  70                  75                  80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
                85                  90                  95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
            100                 105                 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
        115                 120                 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
    130                 135                 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145                 150                 155                 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
                165                 170                 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
            180                 185                 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
        195                 200                 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
    210                 215                 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225                 230                 235                 240
```

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
275 280 285

Asp Gln Pro Ile
290

<210> 29
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 29

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
50 55 60

Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65 70 75 80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
85 90 95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
100 105 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
130 135 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145 150 155 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
165 170 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
180 185 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
195 200 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225 230 235 240

Leu Gly Pro Lys Glu Lys Asp Val Glu Lys Pro Val Asn Asn Pro Phe
245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
275 280 285

Asp Gln Pro Ile
290

<210> 30
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 30

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Lys Ala Tyr Trp Phe Tyr Arg
20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
50 55 60

```
Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65                  70                  75                  80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
                85                  90                  95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
            100                 105                 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
        115                 120                 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
    130                 135                 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145                 150                 155                 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
                165                 170                 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
            180                 185                 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
        195                 200                 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
    210                 215                 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225                 230                 235                 240

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
                245                 250                 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
            260                 265                 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
        275                 280                 285

Asp Gln Pro Ile
    290
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<210> 31
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 31

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Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1               5                   10                  15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
            20                  25                  30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr
        35                  40                  45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
    50                  55                  60

Asp Met Arg Val Val Asn Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65                  70                  75                  80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
                85                  90                  95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
            100                 105                 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
        115                 120                 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
    130                 135                 140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145                 150                 155                 160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
                165                 170                 175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
            180                 185                 190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
        195                 200                 205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
```

210 215 220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225 230 235 240

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
245 250 255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
260 265 270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
275 280 285

Asp Gln Pro Ile
290

<210> 32
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 32

Ala Thr Arg Cys Ser Ser Ser Ser Val Ser Ser Ser Arg Pro Ser Ala
1 5 10 15

Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr Arg
20 25 30

Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Ile
35 40 45

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His Pro
50 55 60

Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
65 70 75 80

Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp Gln
85 90 95

Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu
100 105 110

Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp
115 120 125

Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
    130   135   140

Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly Gly
145   150   155   160

Lys Ala Cys Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg
                165   170   175

Phe Phe Ser Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile
            180   185   190

Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile
        195   200   205

Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
    210   215   220

Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln
225   230   235   240

Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro Phe
                245   250   255

Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp Phe
            260   265   270

Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
        275   280   285

Asp Gln Pro Ile
    290

<210> 33
<211> 293
<212> PRT
<213> Arabidopsis thaliana

<400> 33

Ala Thr Arg Cys Ser Ser Ser Ser Ser Val Ser Ala Ser Arg Pro Ser
1   5   10   15

Ala Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Tyr Trp Phe Tyr
            20   25   30

Arg Phe Leu Ser Ile Val Tyr Asp His Ile Ile Asn Pro Gly His Trp

35 40 45

Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Ser His
50 55 60

Pro Asp Met Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr
65 70 75 80

Leu Gly Ile Val Lys Thr Val Lys Ala Lys Asn Val Thr Ile Leu Asp
85 90 95

Gln Ser Pro His Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys
100 105 110

Glu Cys Lys Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr
115 120 125

Asp Tyr Ala Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro
130 135 140

Asp Pro Gln Arg Gly Ile Arg Glu Ala Tyr Arg Val Leu Lys Ile Gly
145 150 155 160

Gly Lys Ala Cys Leu Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser
165 170 175

Arg Phe Phe Ala Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr
180 185 190

Ile Glu Trp Phe Lys Asn Ala Gly Phe Lys Asp Val Gln Leu Lys Arg
195 200 205

Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met
210 215 220

Gly Cys Ser Val Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu
225 230 235 240

Gln Leu Gly Pro Lys Glu Glu Asp Val Glu Lys Pro Val Asn Asn Pro
245 250 255

Phe Ser Phe Leu Gly Arg Phe Leu Leu Gly Thr Leu Ala Ala Ala Trp
260 265 270

Phe Val Leu Ile Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro
275 280 285

Lys Asp Gln Pro Ile
290

<210> 34
<211> 292
<212> PRT
<213> Arabidopsis thaliana

<400> 34

Arg Leu Arg Cys Ala Ala Ser Ser Ser Ser Ala Ala Ala Arg Pro Met
1 5 10 15

Ser Gln Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr
20 25 30

Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp
35 40 45

Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Tyr Ser
50 55 60

Arg Lys Leu Arg Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr
65 70 75 80

Leu Gly Ile Val Lys Arg Val Asp Pro Glu Asn Val Thr Leu Leu Asp
85 90 95

Gln Ser Pro His Gln Leu Glu Lys Ala Arg Glu Lys Glu Ala Leu Lys
100 105 110

Gly Val Thr Ile Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr
115 120 125

Asp Thr Phe Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro
130 135 140

Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Leu Gly
145 150 155 160

Gly Val Ala Cys Met Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser
165 170 175

Arg Phe Phe Ala Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr
180 185 190

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Ile Glu Trp Phe Lys Lys Ala Gly Phe Lys Asp Val Lys Leu Lys Arg
        195                 200                 205

Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met
    210                 215                 220

Gly Cys Ser Val Thr Gly Val Lys Arg Glu His Gly Asp Ser Pro Leu
225                 230                 235                 240

Gln Leu Gly Pro Lys Val Glu Asp Val Ser Lys Pro Val Asn Pro Ile
                245                 250                 255

Thr Phe Leu Phe Arg Phe Leu Met Gly Thr Ile Cys Ala Ala Tyr Tyr
            260                 265                 270

Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
        275                 280                 285

Gly Met Pro Ile
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<212>  PRT
<213>  Arabidopsis thaliana

<400>  35

Arg Leu Arg Cys Ala Ala Ser Ser Ser Pro Ala Ala Ala Arg Pro Ala
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Thr Ala Pro Arg Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr
            20                  25                  30

Arg Phe Leu Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp
        35                  40                  45

Thr Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Phe Ser
    50                  55                  60

Arg His Leu Thr Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr
65                  70                  75                  80

Leu Gly Ile Val Lys His Val Asn Pro Glu Asn Val Thr Leu Leu Asp
                85                  90                  95

Gln Ser Pro His Gln Leu Asp Lys Ala Arg Gln Lys Glu Ala Leu Lys
            100                 105                 110
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Gly Val Thr Ile Met Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr
115 120 125

Asp Ser Phe Asp Arg Tyr Ile Ser Ala Gly Ser Ile Glu Tyr Trp Pro
130 135 140

Asp Pro Gln Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Arg Phe Gly
145 150 155 160

Gly Leu Ala Cys Val Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser
165 170 175

Arg Phe Phe Ala Asp Met Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr
180 185 190

Ile Glu Trp Phe Lys Lys Ala Gly Phe Arg Asp Val Lys Leu Lys Arg
195 200 205

Ile Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met
210 215 220

Gly Cys Ser Val Thr Gly Val Lys Arg Glu Arg Gly Asp Ser Pro Leu
225 230 235 240

Glu Leu Gly Pro Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Ile
245 250 255

Thr Phe Leu Phe Arg Phe Leu Val Gly Thr Ile Cys Ala Ala Tyr Tyr
260 265 270

Val Leu Val Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys
275 280 285

Gly Met Pro Ile
290

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<211> 288
<212> PRT
<213> Arabidopsis thaliana

<400> 36

Val Pro Lys Cys Ser Val Ser Ala Ser Arg Pro Ser Ser Gln Pro Arg
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Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
35 40 45

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn Asp Arg Asn Met Ile
50 55 60

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
65 70 75 80

Lys His Val Asp Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
85 90 95

Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Lys Ile
100 105 110

Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Arg Thr Asp Tyr Ala Asp
115 120 125

Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
130 135 140

Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Leu Gly Gly Lys Ala Cys
145 150 155 160

Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
165 170 175

Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
180 185 190

Gln Lys Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
195 200 205

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
210 215 220

Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
225 230 235 240

Lys Glu Glu Asp Val Glu Lys Pro Val Asn Pro Phe Val Phe Ala Leu
245 250 255

Arg Phe Val Leu Gly Ala Leu Ala Ala Thr Trp Phe Val Leu Val Pro

260 265 270

Ile Tyr Met Trp Leu Lys Asp Gln Val Val Pro Lys Gly Gln Pro Ile
275 280 285

<210> 37
<211> 289
<212> PRT
<213> Arabidopsis thaliana

<400> 37

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Ser Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp
35 40 45

Met Arg Asp Asp Ala Leu Glu Pro Ala Glu Leu Tyr Asp Ser Arg Met
50 55 60

Lys Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile
65 70 75 80

Ile Lys His Ile Asp Pro Lys Asn Val Thr Ile Leu Asp Gln Ser Pro
85 90 95

His Gln Leu Glu Lys Ala Arg Gln Lys Glu Ala Leu Lys Glu Cys Thr
100 105 110

Ile Val Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Thr Phe
115 120 125

Asp Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln
130 135 140

Arg Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Leu Gly Gly Val Ala
145 150 155 160

Cys Leu Ile Gly Pro Val His Pro Thr Phe Trp Leu Ser Arg Phe Phe
165 170 175

Ala Asp Met Trp Met Leu Phe Pro Thr Glu Glu Glu Tyr Ile Glu Trp
180 185 190

Phe Lys Lys Ala Gly Phe Lys Asp Val Lys Leu Lys Arg Ile Gly Pro
195 200 205

Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser
210 215 220

Val Thr Gly Val Lys Arg Leu Ser Gly Asp Ser Pro Leu Gln Leu Gly
225 230 235 240

Pro Lys Ala Glu Asp Val Lys Lys Pro Ile Asn Pro Phe Ser Phe Leu
245 250 255

Leu Arg Phe Ile Leu Gly Thr Ile Ala Ala Thr Tyr Tyr Val Leu Val
260 265 270

Pro Ile Tyr Met Trp Ile Lys Asp Gln Ile Val Pro Lys Gly Gln Pro
275 280 285

Ile

<210> 38
<211> 288
<212> PRT
<213> Arabidopsis thaliana

<400> 38

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Phe Ile Gln His Lys Lys Glu Ala Phe Trp Phe Tyr Arg Phe Leu Ser
20 25 30

Ile Val Tyr Asp His Val Ile Asn Pro Gly His Trp Thr Glu Asp Met
35 40 45

Arg Asp Asp Ala Leu Glu Pro Ala Asp Leu Asn Asp Arg Asp Met Val
50 55 60

Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu Gly Ile Val
65 70 75 80

Gln His Val Asp Ala Lys Asn Val Thr Ile Leu Asp Gln Ser Pro His
85 90 95

Gln Leu Ala Lys Ala Lys Gln Lys Glu Pro Leu Lys Glu Cys Asn Ile

100 105 110

Ile Glu Gly Asp Ala Glu Asp Leu Pro Phe Pro Thr Asp Tyr Ala Asp
115 120 125

Arg Tyr Val Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp Pro Gln Arg
130 135 140

Gly Ile Lys Glu Ala Tyr Arg Val Leu Lys Gln Gly Gly Lys Ala Cys
145 150 155 160

Leu Ile Gly Pro Val Tyr Pro Thr Phe Trp Leu Ser Arg Phe Phe Ala
165 170 175

Asp Val Trp Met Leu Phe Pro Lys Glu Glu Glu Tyr Ile Glu Trp Phe
180 185 190

Glu Lys Ala Gly Phe Lys Asp Val Gln Leu Lys Arg Ile Gly Pro Lys
195 200 205

Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly Cys Ser Val
210 215 220

Thr Gly Val Lys Pro Ala Ser Gly Asp Ser Pro Leu Gln Leu Gly Pro
225 230 235 240

Lys Ala Glu Asp Val Ser Lys Pro Val Asn Pro Phe Val Phe Leu Leu
245 250 255

Arg Phe Met Leu Gly Ala Thr Ala Ala Ala Tyr Tyr Val Leu Val Pro
260 265 270

Ile Tyr Met Trp Leu Lys Asp Gln Ile Val Pro Glu Gly Gln Pro Ile
275 280 285

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<211> 1047
<212> DNA
<213> Arabidopsis thaliana

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atgacgacaa cgcgtggaaa cgtggctgtg gcggctgctg ctacatccac tgaggcgcta 180

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gatcatatgc atcatggctt ttatgaccct gattcttctg ttcaactttc tgattctggt 300

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atcatcactt gccagaagcc actctaa 1047

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<211> 1047
<212> DNA
<213> Arabidopsis thaliana

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caggccaaga gagccaatga tctcgcggct gctcaatcac tcgctcataa ggcttccttc 540

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gcggctccag gaggtaggat aataatagtg acatggtgcc atagaaatct atctgcgggg 720

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atcatcactt gccagaagcc actctaa 1047

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<211> 1095
<212> DNA
<213> Arabidopsis thaliana

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aaaagtgtag ttgatgttgg ctgtggcatt ggtggtagct caagatactt ggcgaacaaa 480

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ctcgcggcag agcaagggtt atcagacaag gtgcgtattc aagttggtga tgcattggag 600

cagccttttc ctgatgggca gtttgatctt gtctggtcca tggagagtgg cgagcacatg 660

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<211> 1059
<212> DNA
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<211> 1038
<212> DNA
<213> Arabidopsis thaliana

<400> 43

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<211> 1131
<212> DNA
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<212> DNA
<213> Arabidopsis thaliana

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cccatccaga agcagaccca ttccctaact aatcattttt atgaacaatt tgtaacacta 660

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<211> 933
<212> DNA
<213> Arabidopsis thaliana

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gaagatattt ggggtgacca tatgcatcat ggatattatg aacctaaatc ctctgtggaa 180

ctttcagatc atcgtgctgc tcagatccgt atgattgaac aggctctaag ttttgctgct 240

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cctgtacaag cagagagggc tcaagctctt gctgatgctc aaggattagg tgataaggtt 420

tcatttcaag tagcagacgc cttgaatcag ccttttccag atgggcaatt cgacttggtt 480

tggtccatgg agagtggaga acacatgccg aacaaagaaa agtttgttgg cgaattagct 540

cgagtggcag caccaggagg cacaatcatc cttgtcacat ggtgccacag ggacctttcc 600

ccttcggagg aatctctgac tccagaggag aaagagctgt taaataagat atgcaaagcc 660

ttctatcttc cggcttggtg ttccactgct gattatgtga agttacttca atccaattct 720

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atcaaagctg cactggcaat gccactgatg attgaaggat acaagaaagg tctcatcaaa 900

tttgccatca tcacatgtcg aaaacctgaa taa 933

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<211> 909
<212> DNA
<213> Arabidopsis thaliana

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cacggctttt atgacccgga ttccaccgtt tctgtttctg atcatcgcgc tgctcagatc 180

cgaatgatcc aagaatctct tcgttttgcc tctctgcttt ctgagaaccc ttctaaatgg 240

cccaagagta tagttgatgt tgggtgtggc ataggggggca gctccagata cctggccaag 300

aaatttggag caacgagcgt aggcattact ctgagtcctg ttcaagctca aagagcaaat 360

gctcttgctg ctgctcaagg attggctgat aaggtttcct ttcaggttgc tgacgctcta 420
cagcaaccat tctctgacgg ccagtttgat ctggtgtggt ccatggagag tggagagcat 480
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<210> 49
<211> 1053
<212> DNA
<213> Arabidopsis thaliana

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gcatcggcag cgagctcgga gagaggggag atagtattgg agcagaagcc gaagaaggag 180
gaggagggga aactgcagaa gggaatcgca gagttctacg acgagtcgtc tggcttatgg 240
gagaacattt ggggcgacca catgcaccat ggcttttatg acccggattc cactgtttct 300
gtttctgatc atcgcgctgc tcagatccga atgatccaag agtctcttcg ctttgcctct 360
gtttctgagg agcgtagtaa atggcccaag agtatagttg atgttgggtg tggcataggt 420
ggcagctcca gatacctggc caagaaattt ggagcaacca gcgtaggcat tactctgagt 480
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tcctttcagg ttgctgacgc tctacagcaa ccattctctg acggccagtt tgatctggtg 600
tggtccatgg agagtggaga gcatatgcct gacaaagcta agtttgttgg agagttagct 660
cgggtagcag caccaggtgc cactataata atagtaacat ggtgccacag ggatcttggc 720
cctgacgaac aatccttaca tccatgggag caagatctct taaagaagat ttgcgatgca 780
tattaccttc ctgcctggtg ctcaacttct gattatgtta agttgctcca atccctgtca 840
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atacgctcag ccttcacatg gaagggtcta acttcactct tgagcagtgg acttaaaacc 960
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tttgccatca ttacatgtcg aaaacctgaa taa 1053

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<211> 1053
<212> DNA
<213> Arabidopsis thaliana

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caatcccctc gcactttcgc cagaatccgg gtcggaccca ggtcgtgggc tcctattcgg 120

gcatcggcag cgagctcgga gagaggggag atagtattgg agcagaagcc gaagaaggat 180

gacaaggaga aactgcagaa gggaatcgca gagttttacg acgagtcttc tggcttatgg 240

gagaacattt ggggcgacca catgcaccat ggcttttatg acccggattc cactgtttcg 300

ctttcggatc atcgtgctgc tcagatccga atgatccaag agtctcttcg ctttgcctct 360

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ggcagctcca gatacctggc caagaaattt ggagcaacca gtgtaggcat cactctgagt 480

cctgttcaag ctcaaagagc aaatgctctt gctgctgctc aaggattggc tgataaggtt 540

tcctttcagg ttgctgacgc tctacagcaa ccattctctg acggccagtt tgatctggtg 600

tggtccatgg agagtggaga gcatatgcct gacaaagcta agtttgttgg agagttagct 660

cgggtagcag caccaggtgc cactataata atagtaacat ggtgccacag ggatcttggc 720

cctgacgaac aatccttaca tccatgggag caagatctct taaagaagat ttgcgatgca 780

tattacctcc ctgcctggtg ctcaacttct gattatgtta agttgctcca atccctgtca 840

cttcaggaca tcaagtcaga agattggtct cgctttggtg ctccattttg gccagcagtg 900

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ataaaaggag ctttggctat gccattgatg atagagggat acaagaaaga tctaattaag 1020

tttgccatca ttacatgtcg aaaacctgaa taa 1053

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<211> 933
<212> DNA
<213> Arabidopsis thaliana

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gaggatgtgg agctgaagaa aggaattgca gagttctacg atgaatcgtc ggagatgtgg 120

gagaatatat ggggagaaca catgcatcat ggatactata acactaatgc cgttgttgaa 180

ctctccgatc atcgttctgc tcagatccgt atgattgaac aagccctact tttcgcatct 240

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<212> DNA
<213> Sorghum bicolor

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<211> 843
<212> DNA
<213> Arabidopsis thaliana

<400> 53
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aaagaccgcc gtcaggctca aattgattta atcgaagaat tgcttaattg ggcaggggta 180

caagcagcag aagatatact agatgtgggt tgtggaattg gcggtagttc tttatacctg 240

gcgcaaaagt ttaatgctaa agctacaggg attacattga gtcctgtaca agctgcaaga 300

gcaacagaac gcgcattgga agctaatttg agtctgagaa cacagttcca agtcgctaat 360

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gatgaggaaa agcacttgca ggatatttat cgggtgtatt gtttgcctta tgtgatttct 600

ttgccagagt atgaagcgat cgcacatcaa ctaccattac ataatatccg cactgctgat 660

tggtcaactg ctgtcgcccc cttttggaat gtggtaattg attctgcatt cactccccaa 720

gcgctttggg gtttactaaa tgctggttgg actaccattc aaggggcatt atcactggga 780

ttaatgcgtc gcggttatga acgtgggtta attcggtttg gcttactgtg cggcaataag 840

tag 843

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gaagagattt ggggcgaaca tatgcaccac ggctattatg gtgcagacgg tactgaacaa 120

aaaaaccgcc gtcaggcgca aattgattta attgaagaat tactcacttg ggcaggagta 180

caaacagcag aaaatatact agatgtgggt tgtggtattg gtggtagttc tctgtatttg 240

gcaggaaagt tgaatgctaa agctacagga attaccctga gtccagtgca agccgctaga 300

gccacagaaa gagccaagga agctggttta agtggtagaa gtcagttttt agtggcaaat 360

gcccaagcaa tgcctttttga tgataattct tttgacttgg tgtggtcgct agaaagtggc 420

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ggtaagttaa tcatggtgac atggtgtcat cgtcccactg ataaaacacc actgacggct 540

gatgaaaaaa aacacctaga agatatttat cgggtgtatt gtttgcctta tgtaatttcg 600

ttgccggagt atgaagcgat cgcacgtcaa ctaccattaa ataatatccg caccgccgac 660

tggtcgcaat ccgtcgccca attttggaac atagtcatcg attccgcctt taccccccaa 720

gcaatattcg gcttactccg cgcaggttgg actaccatcc aaggagcctt atcactaggc 780

ttaatgcgtc gcggctatga gcgcgggtta attcggtttg ggttgctttg tggggataag 840

tga 843

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<211> 40
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<213> Arabidopsis thaliana

<400> 55
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<210> 56
<211> 40
<212> DNA
<213> Arabidopsis thaliana

<400> 56
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<210> 57
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 57
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<210> 58
<211> 38
<212> DNA
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<400> 58
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<210> 59
<211> 38
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<400> 59
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<210> 60
<211> 38
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<210> 61
<211> 39
<212> DNA
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<400> 61
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<210> 62
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<212> DNA
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<210> 63
<211> 38
<212> DNA
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<400> 63
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<210> 64
<211> 38
<212> DNA
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<400> 64
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<210> 65
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<400> 65

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<210> 66
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<213> Arabidopsis thaliana

<400> 66
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<210> 67
<211> 38
<212> DNA
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<400> 67
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<210> 68
<211> 38
<212> DNA
<213> Arabidopsis thaliana

<400> 68
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<210> 69
<211> 38
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<400> 69
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<211> 38
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<210> 71
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<210> 72
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<210> 73
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<210> 74
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<210> 75
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<210> 76
<211> 38
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<210> 77
<211> 74
<212> DNA
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agcagcagca gcag 74

<210> 78
<211> 62
<212> DNA
<213> Arabidopsis thaliana

<400> 78
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cg 62

<210> 79
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<212> DNA
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<400> 79
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gcggcgtcgt cg 72

<210> 80
<211> 61
<212> DNA
<213> Arabidopsis thaliana

<400> 80
ggggaccact ttgtacaaga aagctgggtc ctgcaggtta gatcggcatg cctttgggca 60

c 61

<210> 81
<211> 72
<212> DNA
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<400> 81
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gcggcgtcgt cg 72

<210> 82
<211> 62
<212> DNA
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<400> 82
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cg 62

<210> 83
<211> 71
<212> DNA
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agtgtctcgg c 71

<210> 84
<211> 61
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<213> Arabidopsis thaliana

<400> 84
ggggaccact ttgtacaaga aagctgggtc ctgcaggtta gattggctga cctttgggaa 60

c 61

<210> 85
<211> 70
<212> DNA
<213> Arabidopsis thaliana

<400> 85
gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatgat ctttacatgc 60

agcgcgtcct 70

<210> 86
<211> 61
<212> DNA
<213> Arabidopsis thaliana

<400> 86
ggggaccact ttgtacaaga aagctgggtc ctgcaggtca tatgggctgg cctttcggta 60

c 61

<210> 87
<211> 72
<212> DNA
<213> Arabidopsis thaliana

<400> 87
gggacaagtt tgtacaaaaa agcaggctta gaaggagata gaaccatggc cccgaggtgc 60

agcttatcag cg 72

<210> 88
<211> 61
<212> DNA
<213> Arabidopsis thaliana

<400> 88
ggggaccact ttgtacaaga aagctgggtc ctgcaggtta gattggttga ccctctggta 60

c 61

<210> 89
<211> 65
<212> DNA
<213> Arabidopsis thaliana

<400> 89
ggggacaagt ttgtacaaaa aagcaggctg cggccgctga acaatggcct ctttgatgct 60

caacg 65

<210> 90
<211> 62
<212> DNA
<213> Arabidopsis thaliana

<400> 90
ggggaccact ttgtacaaga aagctgggtc ctgcaggtca gatgggttgg tctttgggaa 60

cg 62

<210> 91
<211> 348
<212> PRT
<213> Arabidopsis thaliana

<400> 91

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
20 25 30

Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
35 40 45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
50 55 60

Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
65 70 75 80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
85 90 95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
100 105 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
115 120 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
130 135 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
145 150 155 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His
165 170 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
180 185 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
195 200 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
210 215 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
225 230 235 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
245 250 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
260 265 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser
275 280 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
290 295 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
305 310 315 320

Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
325 330 335

Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
340 345

<210> 92
<211> 348
<212> PRT
<213> Arabidopsis thaliana

<400> 92

Met Lys Ala Thr Leu Ala Ala Pro Ser Ser Leu Thr Ser Leu Pro Tyr
1 5 10 15

Arg Thr Asn Ser Ser Phe Gly Ser Lys Ser Ser Leu Leu Phe Arg Ser
20 25 30

```
Pro Ser Ser Ser Ser Ser Val Ser Met Thr Thr Thr Arg Gly Asn Val
        35                  40                  45

Ala Val Ala Ala Ala Ala Thr Ser Thr Glu Ala Leu Arg Lys Gly Ile
    50                  55                  60

Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp Gly
65                  70                  75                  80

Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln Leu
                85                  90                  95

Ser Asp Ser Gly His Lys Glu Ala Gln Ile Arg Met Ile Glu Glu Ser
            100                 105                 110

Leu Arg Phe Ala Gly Val Thr Asp Glu Glu Glu Glu Lys Lys Ile Lys
        115                 120                 125

Lys Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu
    130                 135                 140

Ala Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val
145                 150                 155                 160

Gln Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ala His
                165                 170                 175

Lys Ala Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp
            180                 185                 190

Gly Lys Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro
        195                 200                 205

Asp Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly
    210                 215                 220

Gly Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Ala Gly
225                 230                 235                 240

Glu Glu Ala Leu Gln Pro Trp Glu Gln Asn Ile Leu Asp Lys Ile Cys
                245                 250                 255

Lys Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Asp Asp Tyr Val Asn
            260                 265                 270

Leu Leu Gln Ser His Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser
```

275 280 285

Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr
290 295 300

Trp Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys
305 310 315 320

Gly Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val
325 330 335

Ile Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
340 345

<210> 93
<211> 364
<212> PRT
<213> Oryza sativa

<400> 93

Met Ala His Ala Ala Ala Ala Thr Gly Ala Leu Ala Pro Leu His Pro
1 5 10 15

Leu Leu Arg Cys Thr Ser Arg His Leu Cys Ala Ser Ala Ser Pro Arg
20 25 30

Ala Gly Leu Cys Leu His His His Arg Arg Arg Arg Arg Ser Ser Arg
35 40 45

Arg Thr Lys Leu Ala Val Arg Ala Met Ala Pro Thr Leu Ser Ser Ser
50 55 60

Ser Thr Ala Ala Ala Ala Pro Pro Gly Leu Lys Glu Gly Ile Ala Gly
65 70 75 80

Leu Tyr Asp Glu Ser Ser Gly Val Trp Glu Ser Ile Trp Gly Glu His
85 90 95

Met His His Gly Phe Tyr Asp Ala Gly Glu Ala Ala Ser Met Ser Asp
100 105 110

His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu Ser Leu Ala Phe Ala
115 120 125

Ala Val Pro Gly Ala Asp Asp Ala Glu Lys Lys Pro Lys Ser Val Val
130 135 140

Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Asn Lys
145 150 155 160

Tyr Gly Ala Gln Cys Tyr Gly Ile Thr Leu Ser Pro Val Gln Ala Glu
165 170 175

Arg Gly Asn Ala Leu Ala Ala Glu Gln Gly Leu Ser Asp Lys Val Arg
180 185 190

Ile Gln Val Gly Asp Ala Leu Glu Gln Pro Phe Pro Asp Gly Gln Phe
195 200 205

Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Arg
210 215 220

Gln Phe Val Ser Glu Leu Ala Arg Val Ala Ala Pro Gly Ala Arg Ile
225 230 235 240

Ile Ile Val Thr Trp Cys His Arg Asn Leu Glu Pro Ser Glu Glu Ser
245 250 255

Leu Lys Pro Asp Glu Leu Asn Leu Leu Lys Arg Ile Cys Asp Ala Tyr
260 265 270

Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr Val Lys Ile Ala Glu
275 280 285

Ser Leu Ser Leu Glu Asp Ile Arg Thr Ala Asp Trp Ser Glu Asn Val
290 295 300

Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly
305 310 315 320

Leu Thr Ser Leu Leu Arg Ser Gly Trp Lys Thr Ile Arg Gly Ala Met
325 330 335

Val Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe
340 345 350

Thr Ile Ile Thr Cys Arg Lys Pro Glu Thr Thr Gln
355 360

<210> 94
<211> 352
<212> PRT
<213> Zea mays

<400> 94

```
Met Ala His Ala Ala Leu Leu His Cys Ser Gln Ser Ser Arg Ser Leu
1               5                   10                  15

Ala Ala Cys Arg Arg Gly Ser His Tyr Arg Ala Pro Ser His Val Pro
            20                  25                  30

Arg His Ser Arg Arg Leu Arg Arg Ala Val Val Ser Leu Arg Pro Met
        35                  40                  45

Ala Ser Ser Thr Ala Gln Ala Pro Ala Thr Ala Pro Pro Gly Leu Lys
    50                  55                  60

Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp Glu Asn
65                  70                  75                  80

Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Ser Glu Ala
                85                  90                  95

Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile Glu Glu
            100                 105                 110

Ala Leu Ala Phe Ala Gly Val Pro Ala Ser Asp Asp Pro Glu Lys Thr
        115                 120                 125

Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
    130                 135                 140

Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Thr Gly Ile Thr Leu Ser
145                 150                 155                 160

Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Ala Ala Gln Gly Leu
                165                 170                 175

Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln Pro Phe
            180                 185                 190

Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
        195                 200                 205

Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val Ala Ala
    210                 215                 220

Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Asp
225                 230                 235                 240
```

```
Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu Arg Arg
                245                 250                 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser Asp Tyr
            260                 265                 270

Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Thr Ala Asp
        275                 280                 285

Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys Ser Ala
    290                 295                 300

Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Thr Thr Gly Trp Lys Thr
305                 310                 315                 320

Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr Lys Lys
                325                 330                 335

Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly Ala Ala
            340                 345                 350
```

<210> 95
<211> 345
<212> PRT
<213> Gossypium hirsutum

<400> 95

```
Met Ala Ala Ala Leu Gln Leu Gln Thr His Pro Cys Phe His Gly Thr
1               5                   10                  15

Cys Gln Leu Ser Pro Pro Pro Arg Pro Ser Val Ser Phe Pro Ser Ser
            20                  25                  30

Ser Arg Ser Phe Pro Ser Ser Arg Arg Ser Leu Ser Ala His Val Lys
        35                  40                  45

Ala Ala Ala Ser Ser Leu Ser Thr Thr Thr Leu Gln Glu Gly Ile Ala
    50                  55                  60

Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp
65                  70                  75                  80

His Met His His Gly Tyr Tyr Glu Pro Gly Ser Asp Ile Ser Gly Ser
                85                  90                  95
```

```
Asp His Arg Ala Ala Gln Ile Arg Met Val Glu Glu Ser Leu Arg Phe
            100                 105                 110

Ala Gly Ile Ser Glu Asp Pro Ala Asn Arg Pro Lys Arg Ile Val Asp
        115                 120                 125

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
    130                 135                 140

Gly Ala Lys Cys Gln Gly Ile Thr Leu Ser Pro Val Gln Ala Gly Arg
145                 150                 155                 160

Ala Asn Ala Leu Ala Asn Ala Gln Gly Leu Ala Glu Gln Val Cys Phe
                165                 170                 175

Glu Val Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Asp Gln Phe Asp
            180                 185                 190

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Pro Lys
        195                 200                 205

Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
    210                 215                 220

Val Val Thr Trp Cys His Arg Asp Leu Gly Pro Ser Glu Glu Ser Leu
225                 230                 235                 240

Gln Pro Trp Glu Gln Lys Leu Leu Asn Arg Ile Cys Asp Ala Tyr Tyr
                245                 250                 255

Leu Pro Glu Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Phe Gln Ser
            260                 265                 270

Leu Ser Leu Gln Asp Ile Lys Ala Gly Asp Trp Thr Glu Asn Val Ala
        275                 280                 285

Pro Phe Trp Pro Ala Val Ile Arg Ser Ala Leu Thr Trp Lys Gly Phe
    290                 295                 300

Thr Ser Leu Leu Arg Ser Gly Leu Lys Thr Ile Lys Gly Ala Leu Val
305                 310                 315                 320

Met Pro Leu Met Ile Glu Gly Phe Gln Lys Gly Val Ile Lys Phe Ala
                325                 330                 335

Ile Ile Ala Cys Arg Lys Pro Ala Glu
```

340 345

<210> 96
<211> 376
<212> PRT
<213> cuphea pulcherrima

<400> 96

```
Met Pro Ile Thr Ser Ile Ser Ala Asn Gln Arg Pro Phe Phe Pro Ser
1               5                   10                  15

Pro Tyr Arg Gly Ser Ser Lys Asn Met Ala Pro Pro Glu Leu Ala Gln
            20                  25                  30

Ser Gln Val Pro Met Gly Ser Asn Lys Ser Asn Lys Asn His Gly Leu
        35                  40                  45

Val Gly Ser Val Ser Gly Trp Arg Arg Met Phe Gly Thr Trp Ala Thr
    50                  55                  60

Ala Asp Lys Thr Gln Ser Thr Asp Thr Ser Asn Glu Gly Val Val Ser
65                  70                  75                  80

Tyr Asp Thr Gln Val Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu
                85                  90                  95

Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met His His Gly
            100                 105                 110

Tyr Tyr Asp Gly Ser Thr Pro Val Ser Leu Pro Asp His Arg Ser Ala
        115                 120                 125

Gln Ile Arg Met Ile Asp Glu Ala Leu Arg Phe Ala Ser Val Pro Ser
    130                 135                 140

Gly Glu Glu Asp Glu Ser Lys Ser Lys Ile Pro Lys Arg Ile Val Asp
145                 150                 155                 160

Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Arg Lys Tyr
                165                 170                 175

Gly Ala Glu Cys Arg Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg
            180                 185                 190

Gly Asn Ser Leu Ala Arg Ser Gln Gly Leu Ser Asp Lys Val Ser Phe
        195                 200                 205
```

Gln Val Ala Asp Ala Leu Ala Gln Pro Phe Pro Asp Gly Gln Phe Asp
    210   215   220

Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Ser Lys
225   230   235   240

Phe Val Asn Glu Leu Val Arg Val Ala Ala Pro Gly Gly Thr Ile Ile
                245   250   255

Ile Val Thr Trp Cys His Arg Asp Leu Arg Glu Asp Glu Asp Ala Leu
            260   265   270

Gln Pro Arg Glu Lys Glu Ile Leu Asp Lys Ile Cys Asn Pro Phe Tyr
        275   280   285

Leu Pro Ala Trp Cys Ser Ala Ala Asp Tyr Val Lys Leu Leu Gln Ser
    290   295   300

Leu Asp Val Glu Asp Ile Lys Ser Ala Asp Trp Thr Pro Tyr Val Ala
305   310   315   320

Pro Phe Trp Pro Ala Val Leu Lys Ser Ala Phe Thr Ile Lys Gly Phe
                325   330   335

Val Ser Leu Leu Arg Ser Gly Met Lys Thr Ile Lys Gly Ala Phe Ala
            340   345   350

Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile Lys Phe Ser
        355   360   365

Ile Ile Thr Cys Arg Lys Pro Glu
    370   375

<210> 97
<211> 347
<212> PRT
<213> Brassica napus

<400> 97

Met Lys Ala Thr Leu Ala Pro Ser Ser Leu Ile Ser Leu Pro Arg His
1   5   10   15

Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln Arg
            20   25   30

Pro Ser Ser Ala Leu Met Thr Thr Thr Thr Ala Ser Arg Gly Ser Val

35 40 45

Ala Val Thr Ala Ala Ala Thr Ser Ser Val Glu Ala Leu Arg Glu Gly
50 55 60

Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp
65 70 75 80

Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln
85 90 95

Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu
100 105 110

Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg
115 120 125

Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala
130 135 140

Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln
145 150 155 160

Ala Lys Arg Ala Asn Asp Leu Ala Ala Ala Gln Ser Leu Ser His Lys
165 170 175

Val Ser Phe Gln Val Ala Asp Ala Leu Glu Gln Pro Phe Glu Asp Gly
180 185 190

Ile Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
195 200 205

Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Ala Ala Pro Gly Gly
210 215 220

Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Pro Gly Glu
225 230 235 240

Glu Ala Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys
245 250 255

Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr Val Asp Leu
260 265 270

Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Cys Ala Asp Trp Ser Glu
275 280 285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
290 295 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
305 310 315 320

Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
325 330 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
340 345

<210> 98
<211> 347
<212> PRT
<213> Brassica napus

<400> 98

Met Lys Ala Thr Leu Ala Pro Pro Ser Ser Leu Ile Ser Leu Pro Arg
1 5 10 15

His Lys Val Ser Ser Leu Arg Ser Pro Ser Leu Leu Leu Gln Ser Gln
20 25 30

Arg Arg Ser Ser Ala Leu Met Thr Thr Thr Ala Ser Arg Gly Ser Val
35 40 45

Ala Val Thr Ala Ala Ala Thr Ser Ser Ala Glu Ala Leu Arg Glu Gly
50 55 60

Ile Ala Glu Phe Tyr Asn Glu Thr Ser Gly Leu Trp Glu Glu Ile Trp
65 70 75 80

Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser Ser Val Gln
85 90 95

Leu Ser Asp Ser Gly His Arg Glu Ala Gln Ile Arg Met Ile Glu Glu
100 105 110

Ser Leu Arg Phe Ala Gly Val Thr Glu Glu Glu Lys Lys Ile Lys Arg
115 120 125

Val Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg Tyr Ile Ala
130 135 140

Ser Lys Phe Gly Ala Glu Cys Ile Gly Ile Thr Leu Ser Pro Val Gln
145 150 155 160

Ala Lys Arg Ala Asn Asp Leu Ala Thr Ala Gln Ser Leu Ser His Lys
165 170 175

Val Ser Phe Gln Val Ala Asp Ala Leu Asp Gln Pro Phe Glu Asp Gly
180 185 190

Ile Ser Asp Leu Val Trp Ser Met Glu Ser Gly Glu His Met Pro Asp
195 200 205

Lys Ala Lys Phe Val Lys Glu Leu Val Arg Val Thr Ala Pro Gly Gly
210 215 220

Arg Ile Ile Ile Val Thr Trp Cys His Arg Asn Leu Ser Gln Gly Glu
225 230 235 240

Glu Ser Leu Gln Pro Trp Glu Gln Asn Leu Leu Asp Arg Ile Cys Lys
245 250 255

Thr Phe Tyr Leu Pro Ala Trp Cys Ser Thr Thr Asp Tyr Val Glu Leu
260 265 270

Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Tyr Ala Asp Trp Ser Glu
275 280 285

Asn Val Ala Pro Phe Trp Pro Ala Val Ile Arg Thr Ala Leu Thr Trp
290 295 300

Lys Gly Leu Val Ser Leu Leu Arg Ser Gly Met Lys Ser Ile Lys Gly
305 310 315 320

Ala Leu Thr Met Pro Leu Met Ile Glu Gly Tyr Lys Lys Gly Val Ile
325 330 335

Lys Phe Gly Ile Ile Thr Cys Gln Lys Pro Leu
340 345

<210> 99
<211> 310
<212> PRT
<213> Lycopersicon esculentum

<400> 99

Met Ala Ser Val Ala Ala Met Asn Ala Val Ser Ser Ser Ser Val Glu
1 5 10 15

```
Val Gly Ile Gln Asn Gln Gln Glu Leu Lys Lys Gly Ile Ala Asp Leu
            20                  25                  30

Tyr Asp Glu Ser Ser Gly Ile Trp Glu Asp Ile Trp Gly Asp His Met
        35                  40                  45

His His Gly Tyr Tyr Glu Pro Lys Ser Ser Val Glu Leu Ser Asp His
    50                  55                  60

Arg Ala Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Ser Phe Ala Ala
65                  70                  75                  80

Ile Ser Glu Asp Pro Ala Lys Lys Pro Thr Ser Ile Val Asp Val Gly
                85                  90                  95

Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Gly Ala
            100                 105                 110

Thr Ala Lys Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala Gln
        115                 120                 125

Ala Leu Ala Asp Ala Gln Gly Leu Gly Asp Lys Val Ser Phe Gln Val
    130                 135                 140

Ala Asp Ala Leu Asn Gln Pro Phe Pro Asp Gly Gln Phe Asp Leu Val
145                 150                 155                 160

Trp Ser Met Glu Ser Gly Glu His Met Pro Asn Lys Glu Lys Phe Val
                165                 170                 175

Gly Glu Leu Ala Arg Val Ala Ala Pro Gly Gly Thr Ile Ile Leu Val
            180                 185                 190

Thr Trp Cys His Arg Asp Leu Ser Pro Ser Glu Glu Ser Leu Thr Pro
        195                 200                 205

Glu Glu Lys Glu Leu Leu Asn Lys Ile Cys Lys Ala Phe Tyr Leu Pro
    210                 215                 220

Ala Trp Cys Ser Thr Ala Asp Tyr Val Lys Leu Leu Gln Ser Asn Ser
225                 230                 235                 240

Leu Gln Asp Ile Lys Ala Glu Asp Trp Ser Glu Asn Val Ala Pro Phe
                245                 250                 255
```

Trp Pro Ala Val Ile Lys Ser Ala Leu Thr Trp Lys Gly Phe Thr Ser
260 265 270

Val Leu Arg Ser Gly Trp Lys Thr Ile Lys Ala Ala Leu Ala Met Pro
275 280 285

Leu Met Ile Glu Gly Tyr Lys Lys Gly Leu Ile Lys Phe Ala Ile Ile
290 295 300

Thr Cys Arg Lys Pro Glu
305 310

<210> 100
<211> 302
<212> PRT
<213> GLYCINE MAX

<400> 100

Met Ser Val Glu Gln Lys Ala Ala Gly Lys Glu Glu Glu Gly Lys Leu
1 5 10 15

Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Ile Trp Glu
20 25 30

Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp Ser
35 40 45

Thr Val Ser Val Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile Gln
50 55 60

Glu Ser Leu Arg Phe Ala Ser Leu Leu Ser Glu Asn Pro Ser Lys Trp
65 70 75 80

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
85 90 95

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
100 105 110

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
115 120 125

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
130 135 140

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His

145 150 155 160

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
165 170 175

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
180 185 190

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
195 200 205

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
210 215 220

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
225 230 235 240

Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
245 250 255

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Gln Lys Thr
260 265 270

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
275 280 285

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
290 295 300

<210> 101
<211> 350
<212> PRT
<213> Glycine max

<400> 101

Met Ala Thr Val Val Arg Ile Pro Thr Ile Ser Cys Ile His Ile His
1 5 10 15

Thr Phe Arg Ser Gln Ser Pro Arg Thr Phe Ala Arg Ile Arg Val Gly
20 25 30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
35 40 45

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Glu Glu Glu Gly Lys
50 55 60

```
Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
65                  70                  75                  80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp
                85                  90                  95

Ser Thr Val Ser Val Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
            100                 105                 110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
        115                 120                 125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
    130                 135                 140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
145                 150                 155                 160

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
                165                 170                 175

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
            180                 185                 190

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
        195                 200                 205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
    210                 215                 220

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
225                 230                 235                 240

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
                245                 250                 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
            260                 265                 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
        275                 280                 285

Trp Ser Arg Phe Val Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
    290                 295                 300
```

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Leu Lys Thr
305 310 315 320

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
325 330 335

Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
340 345 350

<210> 102
<211> 350
<212> PRT
<213> Glycine max

<400> 102

Met Ala Thr Val Val Arg Ile Pro Thr Ile Ser Cys Ile His Ile His
1 5 10 15

Thr Phe Arg Ser Gln Ser Pro Arg Thr Phe Ala Arg Ile Arg Val Gly
20 25 30

Pro Arg Ser Trp Ala Pro Ile Arg Ala Ser Ala Ala Ser Ser Glu Arg
35 40 45

Gly Glu Ile Val Leu Glu Gln Lys Pro Lys Lys Asp Asp Lys Glu Lys
50 55 60

Leu Gln Lys Gly Ile Ala Glu Phe Tyr Asp Glu Ser Ser Gly Leu Trp
65 70 75 80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Pro Asp
85 90 95

Ser Thr Val Ser Leu Ser Asp His Arg Ala Ala Gln Ile Arg Met Ile
100 105 110

Gln Glu Ser Leu Arg Phe Ala Ser Val Ser Glu Glu Arg Ser Lys Trp
115 120 125

Pro Lys Ser Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Arg
130 135 140

Tyr Leu Ala Lys Lys Phe Gly Ala Thr Ser Val Gly Ile Thr Leu Ser
145 150 155 160

Pro Val Gln Ala Gln Arg Ala Asn Ala Leu Ala Ala Ala Gln Gly Leu
165 170 175

Ala Asp Lys Val Ser Phe Gln Val Ala Asp Ala Leu Gln Gln Pro Phe
180 185 190

Ser Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly Glu His
195 200 205

Met Pro Asp Lys Ala Lys Phe Val Gly Glu Leu Ala Arg Val Ala Ala
210 215 220

Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys His Arg Asp Leu Gly
225 230 235 240

Pro Asp Glu Gln Ser Leu His Pro Trp Glu Gln Asp Leu Leu Lys Lys
245 250 255

Ile Cys Asp Ala Tyr Tyr Leu Pro Ala Trp Cys Ser Thr Ser Asp Tyr
260 265 270

Val Lys Leu Leu Gln Ser Leu Ser Leu Gln Asp Ile Lys Ser Glu Asp
275 280 285

Trp Ser Arg Phe Gly Ala Pro Phe Trp Pro Ala Val Ile Arg Ser Ala
290 295 300

Phe Thr Trp Lys Gly Leu Thr Ser Leu Leu Ser Ser Gly Gln Lys Thr
305 310 315 320

Ile Lys Gly Ala Leu Ala Met Pro Leu Met Ile Glu Gly Tyr Lys Lys
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Asp Leu Ile Lys Phe Ala Ile Ile Thr Cys Arg Lys Pro Glu
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<212> PRT
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Ala Leu Ser Val Val Ala Ala Glu Val Pro Val Thr Val Thr Pro Ala
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Thr Thr Lys Ala Glu Asp Val Glu Leu Lys Lys Gly Ile Ala Glu Phe
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Tyr Asp Glu Ser Ser Glu Met Trp Glu Asn Ile Trp Gly Glu His Met
        35                  40                  45

His His Gly Tyr Tyr Asn Thr Asn Ala Val Val Glu Leu Ser Asp His
    50                  55                  60

Arg Ser Ala Gln Ile Arg Met Ile Glu Gln Ala Leu Leu Phe Ala Ser
65                  70                  75                  80

Val Ser Asp Asp Pro Val Lys Lys Pro Arg Ser Ile Val Asp Val Gly
                85                  90                  95

Cys Gly Ile Gly Gly Ser Ser Arg Tyr Leu Ala Lys Lys Tyr Glu Ala
            100                 105                 110

Glu Cys His Gly Ile Thr Leu Ser Pro Val Gln Ala Glu Arg Ala Gln
        115                 120                 125

Ala Leu Ala Ala Ala Gln Gly Leu Ala Asp Lys Ala Ser Phe Gln Val
    130                 135                 140

Ala Asp Ala Leu Asp Gln Pro Phe Pro Asp Gly Lys Phe Asp Leu Val
145                 150                 155                 160

Trp Ser Met Glu Ser Gly Glu His Met Pro Asp Lys Leu Lys Phe Val
                165                 170                 175

Ser Glu Leu Val Arg Val Ala Ala Pro Gly Ala Thr Ile Ile Ile Val
            180                 185                 190

Thr Trp Cys His Arg Asp Leu Ser Pro Gly Glu Lys Ser Leu Arg Pro
        195                 200                 205

Asp Glu Glu Lys Ile Leu Lys Lys Ile Cys Ser Ser Phe Tyr Leu Pro
    210                 215                 220

Ala Trp Cys Ser Thr Ser Asp Tyr Val Lys Leu Leu Glu Ser Leu Ser
225                 230                 235                 240

Leu Gln Asp Ile Lys Ala Ala Asp Trp Ser Ala Asn Val Ala Pro Phe
                245                 250                 255

Trp Pro Ala Val Ile Lys Thr Ala Leu Ser Trp Lys Gly Ile Thr Ser
            260                 265                 270

Leu Leu Arg Ser Gly Trp Lys Ser Ile Arg Gly Ala Met Val Met Pro
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Leu Met Ile Glu Gly Phe Lys Lys Asp Ile Ile Lys Phe Ser Ile Ile
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Thr Cys Lys Lys Pro Glu
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Glu Arg Arg Ala Ala Gly Gly Arg Arg Glu Pro Leu Gly Gly Gly Ser
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Val Pro Val Gly Ser His Tyr Tyr Tyr Arg Ala Pro Ser His Val Pro
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Arg Arg Ser Arg Pro Arg Gly Arg Gly Gly Val Val Ser Leu Arg Pro
35 40 45

Met Ala Ser Ser Thr Ala Ala Gln Pro Pro Ala Pro Ala Pro Pro Gly
50 55 60

Leu Lys Glu Gly Ile Ala Gly Leu Tyr Asp Glu Ser Ser Gly Leu Trp
65 70 75 80

Glu Asn Ile Trp Gly Asp His Met His His Gly Phe Tyr Asp Ser Gly
85 90 95

Glu Ala Ala Ser Met Ala Asp His Arg Arg Ala Gln Ile Arg Met Ile
100 105 110

Glu Glu Ala Leu Ala Phe Ala Ala Val Pro Ser Pro Asp Asp Pro Glu
115 120 125

Lys Ala Pro Lys Thr Ile Val Asp Val Gly Cys Gly Ile Gly Gly Ser
130 135 140

Ser Arg Tyr Leu Ala Lys Lys Tyr Gly Ala Gln Cys Lys Gly Ile Thr
145 150 155 160

Leu Ser Pro Val Gln Ala Glu Arg Gly Asn Ala Leu Ala Thr Ala Gln
165 170 175

Gly Leu Ser Asp Gln Val Thr Leu Gln Val Ala Asp Ala Leu Glu Gln
180 185 190

Pro Phe Pro Asp Gly Gln Phe Asp Leu Val Trp Ser Met Glu Ser Gly
195 200 205

Glu His Met Pro Asp Lys Arg Lys Phe Val Ser Glu Leu Ala Arg Val
210 215 220

Ala Ala Pro Gly Gly Thr Ile Ile Ile Val Thr Trp Cys His Arg Asn
225 230 235 240

Leu Glu Pro Ser Glu Thr Ser Leu Lys Pro Asp Glu Leu Ser Leu Leu
245 250 255

Lys Arg Ile Cys Asp Ala Tyr Tyr Leu Pro Asp Trp Cys Ser Pro Ser
260 265 270

Asp Tyr Val Asn Ile Ala Lys Ser Leu Ser Leu Glu Asp Ile Lys Ala
275 280 285

Ala Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala Val Ile Lys
290 295 300

Ser Ala Leu Thr Trp Lys Gly Leu Thr Ser Leu Leu Thr Ser Gly Trp
305 310 315 320

Lys Thr Ile Arg Gly Ala Met Val Met Pro Leu Met Ile Gln Gly Tyr
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Lys Lys Gly Leu Ile Lys Phe Thr Ile Ile Thr Cys Arg Lys Pro Gly
340 345 350

Ala Ala

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<211> 128
<212> PRT
<213> Lilium asiaticum

<400> 105

Glu Ser Gly Glu His Met Pro Asp Lys Thr Lys Phe Val Gly Glu Leu
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Ala Arg Val Ala Ala Pro Gly Ala Thr Ile Ile Ile Val Thr Trp Cys

20 25 30

His Arg Asp Leu Leu Pro Ser Glu Asp Ser Leu Arg Pro Asp Glu Ile
35 40 45

Ser Leu Leu Asn Lys Ile Cys Asp Ala Tyr Tyr Leu Pro Lys Trp Cys
50 55 60

Ser Ala Val Asp Tyr Val Lys Ile Ala Glu Ser Tyr Ser Leu Glu Lys
65 70 75 80

Ile Arg Thr Ala Asp Trp Ser Glu Asn Val Ala Pro Phe Trp Pro Ala
85 90 95

Val Ile Arg Ser Ala Leu Thr Trp Lys Gly Phe Thr Ser Leu Leu Arg
100 105 110

Ser Gly Trp Lys Thr Ile Arg Gly Ala Leu Val Met Pro Leu Met Ile
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<210> 106
<211> 280
<212> PRT
<213> Nostoc punctiforme

<400> 106

Met Ser Ala Thr Leu Tyr Gln Gln Ile Gln Gln Phe Tyr Asp Ala Ser
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Ser Gly Leu Trp Glu Gln Ile Trp Gly Glu His Met His His Gly Tyr
20 25 30

Tyr Gly Ala Asp Gly Thr Gln Lys Lys Asp Arg Arg Gln Ala Gln Ile
35 40 45

Asp Leu Ile Glu Glu Leu Leu Asn Trp Ala Gly Val Gln Ala Ala Glu
50 55 60

Asp Ile Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu
65 70 75 80

Ala Gln Lys Phe Asn Ala Lys Ala Thr Gly Ile Thr Leu Ser Pro Val
85 90 95

Gln Ala Ala Arg Ala Thr Glu Arg Ala Leu Glu Ala Asn Leu Ser Leu
100 105 110

Arg Thr Gln Phe Gln Val Ala Asn Ala Gln Ala Met Pro Phe Ala Asp
        115   120   125

Asp Ser Phe Asp Leu Val Trp Ser Leu Glu Ser Gly Glu His Met Pro
    130   135   140

Asp Lys Thr Lys Phe Leu Gln Glu Cys Tyr Arg Val Leu Lys Pro Gly
145   150   155   160

Gly Lys Leu Ile Met Val Thr Trp Cys His Arg Pro Thr Asp Glu Ser
                165   170   175

Pro Leu Thr Ala Asp Glu Glu Lys His Leu Gln Asp Ile Tyr Arg Val
            180   185   190

Tyr Cys Leu Pro Tyr Val Ile Ser Leu Pro Glu Tyr Glu Ala Ile Ala
        195   200   205

His Gln Leu Pro Leu His Asn Ile Arg Thr Ala Asp Trp Ser Thr Ala
    210   215   220

Val Ala Pro Phe Trp Asn Val Val Ile Asp Ser Ala Phe Thr Pro Gln
225   230   235   240

Ala Leu Trp Gly Leu Leu Asn Ala Gly Trp Thr Thr Ile Gln Gly Ala
                245   250   255

Leu Ser Leu Gly Leu Met Arg Arg Gly Tyr Glu Arg Gly Leu Ile Arg
            260   265   270

Phe Gly Leu Leu Cys Gly Asn Lys
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<210> 107
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<212> PRT
<213> Anabaena sp.

<400> 107

Met Ser Ala Thr Leu Tyr Gln Gln Ile Gln Gln Phe Tyr Asp Ala Ser
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Ser Gly Leu Trp Glu Glu Ile Trp Gly Glu His Met His His Gly Tyr
            20   25   30

Tyr Gly Ala Asp Gly Thr Glu Gln Lys Asn Arg Arg Gln Ala Gln Ile

35 40 45

Asp Leu Ile Glu Glu Leu Leu Thr Trp Ala Gly Val Gln Thr Ala Glu
50 55 60

Asn Ile Leu Asp Val Gly Cys Gly Ile Gly Gly Ser Ser Leu Tyr Leu
65 70 75 80

Ala Gly Lys Leu Asn Ala Lys Ala Thr Gly Ile Thr Leu Ser Pro Val
85 90 95

Gln Ala Ala Arg Ala Thr Glu Arg Ala Lys Glu Ala Gly Leu Ser Gly
100 105 110

Arg Ser Gln Phe Leu Val Ala Asn Ala Gln Ala Met Pro Phe Asp Asp
115 120 125

Asn Ser Phe Asp Leu Val Trp Ser Leu Glu Ser Gly Glu His Met Pro
130 135 140

Asp Lys Thr Lys Phe Leu Gln Glu Cys Tyr Arg Val Leu Lys Pro Gly
145 150 155 160

Gly Lys Leu Ile Met Val Thr Trp Cys His Arg Pro Thr Asp Lys Thr
165 170 175

Pro Leu Thr Ala Asp Glu Lys Lys His Leu Glu Asp Ile Tyr Arg Val
180 185 190

Tyr Cys Leu Pro Tyr Val Ile Ser Leu Pro Glu Tyr Glu Ala Ile Ala
195 200 205

Arg Gln Leu Pro Leu Asn Asn Ile Arg Thr Ala Asp Trp Ser Gln Ser
210 215 220

Val Ala Gln Phe Trp Asn Ile Val Ile Asp Ser Ala Phe Thr Pro Gln
225 230 235 240

Ala Ile Phe Gly Leu Leu Arg Ala Gly Trp Thr Thr Ile Gln Gly Ala
245 250 255

Leu Ser Leu Gly Leu Met Arg Arg Gly Tyr Glu Arg Gly Leu Ile Arg
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Phe Gly Leu Leu Cys Gly Asp Lys
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Cys Xaa Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Arg Pro Xaa Xaa
65 70 75 80

Xaa Pro Arg Phe Ile Gln His Lys Xaa Glu Ala Xaa Trp Phe Tyr Arg
85 90 95

Phe Leu Ser Ile Val Tyr Asp His Xaa Ile Asn Pro Gly His Trp Thr
100 105 110

Glu Asp Met Arg Asp Asp Ala Leu Glu Pro Ala Xaa Leu Xaa Xaa Xaa
115 120 125

Xaa Xaa Xaa Val Val Asp Val Gly Gly Gly Thr Gly Phe Thr Thr Leu
130 135 140

Gly Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Val Thr Xaa Leu Asp Gln
145 150 155 160

Ser Pro His Gln Leu Xaa Lys Ala Xaa Xaa Lys Glu Xaa Leu Lys Xaa
165 170 175

Xaa Xaa Ile Xaa Glu Gly Asp Ala Glu Asp Leu Pro Phe Xaa Thr Asp
180 185 190

Xaa Xaa Asp Arg Tyr Xaa Ser Ala Gly Ser Ile Glu Tyr Trp Pro Asp
195 200 205

Pro Gln Arg Gly Ile Xaa Glu Ala Tyr Arg Val Leu Xaa Xaa Gly Gly
210 215 220

Xaa Ala Cys Xaa Ile Gly Pro Val Xaa Pro Thr Phe Trp Leu Ser Arg
225 230 235 240

Phe Phe Xaa Asp Xaa Trp Met Leu Phe Pro Xaa Glu Glu Glu Tyr Ile
245 250 255

Glu Trp Phe Xaa Xaa Ala Gly Phe Xaa Asp Val Xaa Leu Lys Arg Ile
260 265 270

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Gly Pro Lys Trp Tyr Arg Gly Val Arg Arg His Gly Leu Ile Met Gly
        275                 280                 285

Cys Ser Val Thr Gly Val Lys Xaa Xaa Xaa Gly Asp Ser Pro Leu Xaa
    290                 295                 300

Leu Gly Pro Lys Xaa Glu Asp Val Xaa Lys Pro Xaa Xaa Asn Pro Xaa
305                 310                 315                 320

Xaa Phe Xaa Xaa Arg Phe Xaa Xaa Gly Xaa Xaa Xaa Ala Xaa Xaa Xaa
                325                 330                 335

Val Leu Xaa Pro Ile Tyr Met Trp Xaa Lys Asp Gln Xaa Val Pro Xaa
            340                 345                 350

Xaa Xaa Pro Ile
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